Berkeley Center for Green Chemistry

Greener Solutions

Low-Temperature Oily Soil Removal in Laundry

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Table of Contents

Abbreviations

BCF	Bioconcentration factor
CASRN	Chemical Abstracts Service Registry Number
EPA	Environmental Protection Agency (U.S.)
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
HBN	Healthy Building Network
HSDB	Hazardous Substances Data Bank
HSP	Hansen solubility parameter
NADES	Natural deep eutectic solvents
NLM	National Library of Medicine (U.S.)
OECD	Organization for Economic Co-operation and Development
RSC	Royal Society for Chemistry
UNECE	United Nations Economic Commission for Europe

1. Executive Summary

This report provides an assessment of current laundry detergent formulation and proposes new chemical strategies to enable low temperature oily soil removal in laundry. Our partners in this endeavor included Method and Seventh Generation, who are both seeking industry-wide solutions to this challenge, and Amyris and BioAmber, two companies who are developing innovative, sustainable, and green ways to produce chemicals.

To develop these strategies, we first assessed the functions of ingredients in Method's current laundry detergent formulation, as well as the potential hazards associated with these chemicals. We ascertained azard information from databases such as PubMed, the Hazardous Substances Data Bank, and eChem Portal. We evaluated ingredients based on UN's Global Harmonized System, GreenScreen for Safer Chemicals, and several computational estimation tools. We then used biological inspiration to develop strategies that addressed our challenge on a chemical, formulation, or process level. The strategies we report are inspired by NADES, bio-based solvents, biosurfactants, enzymes, and oil-adhesive surfaces. For each, we describe the inspiration, technical feasibility, design concept, potential risks to human and environmental health, and research gaps and priorities.

We conclude with a summary evaluation of how our strategies compare to current laundry processes regarding environmental and human health hazards. We further make recommendations to our partners for implementation of our strategies based on technical feasibility and remaining data gaps.

2. Introduction

Our partners presented us with a challenge of removing oily soils from laundry at low temperatures. Our primary strategy for exploring solutions to this challenge was via biologically inspired design. Biologically inspired design seeks to mimic nature's products and processes based on their efficiency, parsimony, and low impact on the environment (Biomimicry 3.8 Institute, n.d.). Rather than identify additional synthetic chemicals that aid in low temperature removal of oily compounds from laundry, we sought to emulate substances and processes from nature that already perform this function efficiently and with little to no waste to the environment.

A tremendous amount of energy is used globally to heat water for washing clothes, with approximately 90% of laundry cost expended by heating the water (CEC, 2015). Global trends among consumers and manufacturers in Asia, Latin America, and Europe already aim to lower washing temperatures and water use to make this process more economically and environmentally friendly (Lund, 2010). Low temperature can be defined as unheated water within the range of 40-70 °F (5-20 °C), depending on geographic location.

There exist three classes of oily soils in laundry: body soils secreted from humans, food soils and colored stains, such as kitchen grease and wine or coffee, and organic soils, including dirt and clay particles (Aehle, 2004). These types of soils can agglomerate together based on their affinity for lipids and proteins to form highly complex stains composed of various compounds. They are hydrophobic compounds, which are difficult to remove from laundry. Hot washing temperature aids in the removal of oily soils because it facilitates hydrophobic compounds becoming slightly more soluble in water and being lifted off of textile surfaces. However, the ability to clean oily soils in laundry at lower temperatures in standard washing machines would reduce energy consumption worldwide.

The process of washing clothes includes: washing equipment; materials added, such as the textile wash load, detergents, and water quality; and the washing procedure, including time, temperature and agitation. The three types of energy utilized are chemical, mechanical and thermal (Aehle, 2004). Removing the thermal energy input from hot water requires us to instead rely on chemical and mechanical energy to remove stains. Due to established washing machine technology in developed nations, we did not focus on mechanical energy input, but instead investigated chemical energy input alterations of laundry detergents to remove oily soils.

We considered a hierarchy of scale approach to identify solutions pertaining to distinct levels of washing: chemical level changes, whereby ingredients are directly added to the current formulation; formulation level changes, consisting of changing the current laundry detergent formulation by adding multiple ingredients that perform in conjunction with each other; and process level changes, which require altering the process of cleaning laundry to remove oily soils more effectively. Throughout this report, we identify potential solutions that may be more effective in cleaning oily soils at low temperatures compared to current detergent products, as well as ingredients that are safer for the environment and human health.

3. Methods

We performed human and environmental hazard assessments to evaluate current laundry detergent ingredients. This information served to aid in comparing current ingredients to potential opportunities addressed in the strategies section.

3.1 Chemical identification

We searched for chemicals by name in various databases:

- ChemSpider (RSC) <http://www.chemspider.com/>
- PubChem (NLM) <https://pubchem.ncbi.nlm.nih.gov/>
- ChemIDplus (NLM) <http://chem.sis.nlm.nih.gov/chemidplus/>
- Pharos (HBN) <https://www.pharosproject.net/>
- Google <https://www.google.com/>

3.2 Names and identifiers

We confirmed chemical identifiers by inspecting molecular structures (where possible) in PubChem and ChemSpider and comparing the structures against the primary sources from which the substances were identified (e.g., in academic papers or lists of product ingredients). We retrieved PubChem CIDs and ChemSpider IDs from those two databases directly, and CASRN from any available source. We verified CASRN by looking them up in NLM ChemIDplus Lite, which is a highly curated, authoritative, and free database.

3.3 Chemical structures

Where possible, we used the molecular structure from PubChem (i.e. the structural representation linked to each PubChem CID) as the definitive representation of each compound, unless we believed these structures to be in error. For some chemicals, such as ingredients of current laundry products, structural identification was not possible because of the confidential nature of that information.

3.4 Chemical hazard information

We searched for hazard information associated with each chemical—by name, synonym, CASRN, structure, or other identifier—in all sources named above, plus the following additional sources:

- PubMed (NLM) <http://www.ncbi.nlm.nih.gov/pubmed>
- Hazardous Substances Data Bank (NLM) <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>
- eChemPortal (OECD) <http://echemportal.org/>

3.5 Computational estimation tools

We used EPI Suite (US Environmental Protection Agency, 2012a) and PBT Profiler (US Environmental Protection Agency, 2012b) to obtain estimates of environmental fate, partitioning, persistence, and bioaccumulation properties of chemicals—especially when experimental data concerning these properties were not available. We also used receptor binding scores generated by LASSO (Ligand Activity in Surface Similarity Order), a ligand-based virtual screening tool (SimBioSys, Inc., n.d.), to provide indications of potential biological interactions when experimental toxicity studies were unavailable. EPI Suite and LASSO results are available for selected chemicals in ChemSpider. Since these are estimation tools based on models, we used the results in our evaluations only when they represented proper applications of each model to a substance and property within its domain of applicability. Every model has limitations, but at they can be useful: we used computational results as approximate and imperfect indicators of hazard, but still preferable to no data at all.

3.6 Hazard assessment

We used two unifying frameworks to synthesize disparate sources of chemical hazard information into systematic hazard categorizations, and to allow comparison between different chemicals on the basis of hazard.

First, we used the United Nations' Globally Harmonized System (GHS) to group information by the kind of hazard for which it provides evidence (United Nations Economic Commission for Europe, 2015). Broadly, this system encompasses groupings of health hazards, environmental hazards, and physical hazards. For each particular hazard endpoint (e.g. cancer, acute aquatic toxicity, etc.), GHS provides criteria to categorize the severity of hazard suggested by a given body of scientific evidence. Applying the GHS system results in an array of standardized hazard classifications for each chemical, which are meant to be comparable with each other even if different forms of evidence are used to produce them.

Second, we used the GreenScreen for Safer Chemicals (Clean Production Action, 2014), a widely-adopted system which translates GHS classifications, scientific data, and other forms of evidence into endpoint-specific hazard ratings on a scale of *very low - low - moderate - high - very high.* GreenScreen is helpful to provide a standardized and broadly understandable indication of the overall risk associated with each hazard. While GreenScreen also enables combining all hazard information for each chemical (i.e. about all hazard endpoints) into a single overall “benchmark score,” we did not do this. Benchmarking with GreenScreen requires extensive data and research, and was outside the scope of this project.

3.7 Exposure assessment

We used hazard assessment methods to consider the intrinsic potential for harm attributable to each substance of interest. We also considered the potential for human exposure to those substances as products are intended to be manufactured and used. While we did not conduct a rigorous analysis of exposure scenarios, we were informed by how products are typically used and by certain intrinsic properties of chemicals (e.g. their volatility) that would influence how much exposure people are likely to experience. This exposure assessment guided us in highlighting or giving more (or less) weight to specific indications of intrinsic hazard.

3.8 Bioinspired design

We looked to biologically inspired design to develop strategies that would address our challenge. Specifically, we searched online databases such as AskNature to see what biological processes have previously been translated into design practices (Biomimicry 3.8 Institute, n.d.), and conducted other research on natural processes to inspire new ideas. After researching different ways in which nature cleans dirt or oily substances at lower temperatures, we translated our findings into strategies for laundry applications by emulating chemical aspects of the natural processes that were functionally important.

4. Human and Environmental Hazard Assessment of Current Laundry Ingredients

In this section we summarize our hazard assessment of the chemical ingredients used for cleaning in current laundry formulations. We selected a laundry detergent as a product that we consider representative of leading environmentally-preferred formulations. We analyzed detergent ingredients using chemical characterization, searching, and hazard/exposure assessment methods as described above. In this summary we discuss surfactants, solvents, enzymes, and fragrances that appeared to present the most significant environmental and health concerns of the laundry detergent ingredients we investigated.

4.1 Surfactants

Surfactants currently used include lauryl ethoxylate (LAE), sodium lauryl sulfate (SLS), PEG 600 monooctyl ether, and soy methyl ester ethoxylate (MEE). LAE and PEG 600 monooctyl ether are both polyethers with 3-6 oxyethylene units ($-OCH_2CH_2-$) and a terminal alkyl chain, either lauryl (C_{12}) or octyl (C_8). MEE is not actually a methyl ester (as its trade name suggests), but is the poly(oxyethylene) ester of a proprietary soy-derived fatty acid (i.e., the PEG transesterification product of “soy methyl ester”).

In vitro and/or *in vivo* testing of LAE, SLS, and PEG 600 monooctyl ether indicate no genotoxic, mutagenic or carcinogenic properties (Organisation for Economic Cooperation and Development, 2001, 2005), but they pose some ecotoxic and skin/eye irritation hazards. LAE displays dermal and eye irritancy and damage to mucous membranes (US National Library of Medicine, 2013). Data available in the US EPA ECOTOX database (US Environmental Protection Agency, n.d.) report measured aquatic LC_{50}’s for amphipods and fish in the mg/L range (GreenScreen: high/moderate) and measured BCF in fish (carp) ranging from 40 - 200 L/kg (GreenScreen: low/very low). Environmental fate modeling (EPI Suite) predicts considerable partitioning to soil, water, and sediment.

Few hazard classifications and data sources exist for PEG 600 monooctyl ether, but we infer that it is very similar to LAE based on its structure. We estimate that it has moderate aquatic toxicity and very low BCF based on EPI suite predictions. Sodium lauryl sulfate (SLS) has some aquatic and terrestrial ecotoxicity (GHS categories ranging from 1-3). Since it is anionic, it is also expected to be essentially nonbioaccumulative. SLS shows significant irritant properties and acute toxicity for eyes and skin, cited on New Zealand, Japan, and Canada authoritative lists.

Significant data gaps exist regarding hazard identification of PEG 600 monooctyl ether and especially soy methyl ether ethoxylate (MEE). Due to the proprietary status of MEE, we do not have access to more detailed chemical information.

4.2 Solvents

We examined three solvents currently present in this laundry detergent: glycerol, 1,2-propanediol, and monoisopropanolamine. Glycerol and 1,2-propanediol are short-chain alcohols generally recognized as safe in U.S. food products. For both of these compounds, we found evidence of safety with respect to mutagenic, reproductive, and developmental endpoints. They are also not acutely toxic. Glycerol can cause kidney failure if ingested at high doses, but this is unlikely to be relevant to laundry detergents.

Monoisopropanolamine (1-amino-2-hydroxypropane) has a chemical structure and safety profile similar to the two alcohols above. However, we found a lack of data for cancer, mutagenicity, and reproductive toxicity endpoints. The most significant human health hazard is severe skin burns and eye damage (GreenScreen: *very high* hazard for skin irritation and eye irritation/damage). This may be most relevant for occupational exposures, which are often more intense and frequent.

For all three of these polar protic 3-carbon solvents, ecotoxicity, persistence, and bioaccumulation are very low. They have high boiling points and low vapour pressures, and therefore have very low intrinsic exposure potential by inhalation.

4.3 Enzymes

There are significant data gaps in the hazard identification of the current enzyme ingredients: protease, amylase, and mannanase. Some sources cite a respiratory hazard in occupational or high dose environments due to the sensitizing nature of proteolytic enzymes. A review article in the British Journal of Dermatology estimated very low risk of respiratory sensitization for the consumer using enzymatic laundry detergents due to a typically low dose exposure and limited inhalation risk (D. A. Basketter, English, Wakelin, & White, 2008). If dermal contact occurs with normal use of the laundry detergent, dermal irritation could also be a concern. The Pharos database reports that enzymes used in current laundry detergent products present hazards of dermal and respiratory irritation and sensitization, as well as sensitizer-induced asthma, aquatic toxicity, and ecotoxicity at high concentrations/doses. Although these concerns are important to consider, it is reported that there is limited exposure to the consumer, with a very low dose of enzymes in the detergent (up to 0.8%) (Hasan, Shah, Javed, & Hameed, 2013).

4.4 Fragrances

Although fragrances are not part of our current investigation of the oily soil removal performance of laundry detergents at low temperatures, we make note here of their concerning human and environmental hazards. All fragrances commonly used in the laundry detergent we investigated (Methylpropional, Linalool, Limonene, Hexyl Cinnamal, Amyl Cinnamal, Geraniol, Citonellol, Benzyl salicylate, Butylphenyl methylpropional) present low to high aquatic toxicity as reported in the Pharos database. In addition to high aquatic toxicity, Limonene presents environmental toxicity and persistence, as well as respiratory and dermal irritation and sensitization, developmental toxicity, and potential carcinogenicity. Other fragrances pose potential endocrine disruption and dermal irritation and sensitization as reported in the Pharos database.

5. Strategies Overview

We approached this challenge by considering cleaning at three different levels: the chemical, formulation, and process levels. Chemical level changes consider direct replacement and/or addition of ingredients that are currently used in laundry detergent with ones that we believe might be more effective as well as safe for humans and the environment. Formulation level changes include proposing strategies that impact how laundry detergent ingredients interact with each other. Process level changes approach cleaning on a broader scale, and include more "out of the box" ideas for cleaning stained fabrics at low temperatures. We have developed several strategies that fall somewhere along this spectrum of changes, each of which was either inspired by or derived from nature (Figure 1).

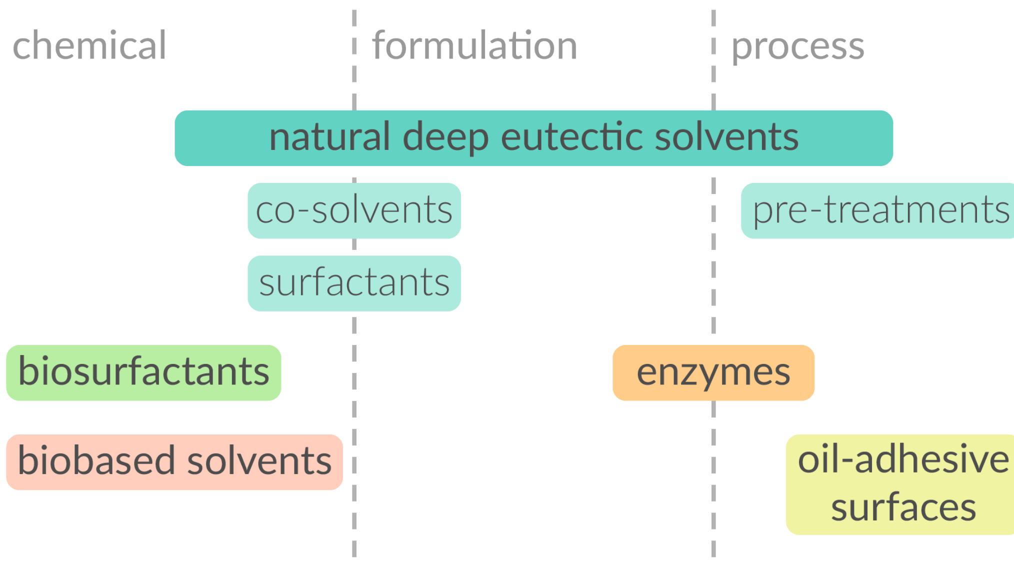


Figure 1. Schematic of our approach for developing solutions to our challenge.

6. NADES

6.1 Inspiration

Natural deep eutectic solvents (NADES)—a relatively new class of compounds first described in 2003—are mixtures of chemicals abundantly found in nature such as sugars, amino acids, and organic acids (Abbott, Capper, Davies, Rasheed, & Tambyrajah, 2003). In nature, they can act as an alternative media to water in biological systems, where they are able to solubilize, store, and transport relatively nonpolar compounds, such as lipids. These solvents may also play an important role for organisms under very cold, dry, or otherwise extreme conditions (Dai, van Spronsen, Witkamp, Verpoorte, & Choi, 2013b).

6.2 Technical Feasibility

6.2.1 Depressed Melting Point

NADES are composite substances whose physical and chemical characteristics differ from those of their component parts. Combining two or more solid chemicals in particular molar ratios produces a liquid - a deep eutectic solvent- with a lower melting temperature than any of its individual components. NADES are often formed by combining and heating chemicals that are solid at room temperature until intermolecular hydrogen bonds form and the mixture liquefies (Francisco, van den Bruinhorst, & Kroon, 2013). The depressed melting point of NADES suggests they may be effective at removing non-water soluble compounds at low temperatures. The specific melting points of various NADES have been determined experimentally and reported in the literature, and they exhibit a wide range of melting points, determined by the chemical make up and molar ratio of their constituents. NADES can exist as solvents in environments between 0 - 100 C, although most deep eutectic solvents have a melting point above 50 C, well above the range considered “low temperature” in this application (Dai, van Spronsen, Witkamp, Verpoorte, & Choi, 2013a). We identified some NADES with melting temperatures in the range of 10-30 degrees C (Table 1)(Dai et al., 2013a). These compounds, their components, or their unique chemical properties may prove useful in anticipating the depressed melting temperature of other NADES that will be proposed for use in laundry.

Table 1. Examples of NADES with melting temperatures relevant to low temperature laundry.

Chemical 1	**Chemical 2**	**Molar ratio**	**Melting pt. (°C)**
glycerol	choline chloride	3:1	20
glycerol	choline chloride	2:1	23
urea	choline chloride	2:1	12

6.2.2 Properties In Aqueous Environments

The hydrogen bonds that give NADES their relatively low melting point also allow for the incorporation of water molecules into the supramolecular NADES structure. The addition of water affects several intrinsic properties of the solvents such as their viscosity, density, conductivity, and polarity. Water content of NADES also directly influences their function as solvents; pure NADES without water are generally good at solubilizing nonpolar solutes. As water content increases, NADES themselves become more polar and thus become more suited to solubilize compounds of medium polarity (Dai, Witkamp, Verpoorte, & Choi, 2015).

The role of water in NADES structure and function is important when considering the feasibility of their application in laundry detergent. Since we cannot change the technology of washing machines and the volume of water used during wash cycles, understanding NADES ability to function as a solvent in high quantities of water is extremely important. High water content could potentially interfere with hydrogen bonds between NADES components, breaking up the solvent into its component parts. In this scenario, we would expect the solvent to lose its unique properties, reverting to an aqueous solution of the component chemicals. Literature reports a range of NADES tolerance for water from decomposition when water content exceeds 50% by volume of the solvent (Dai et al., 2015), to solvents that retain their integrity in aqueous solutions (Wen, Chen, Tang, Wang, & Yang, 2015). Under the latter scenario, though, even if NADES maintain their chemical structure, they may not be able to make highly hydrophobic particulates soluble. More research should elucidate the effects of water content in specific NADES to identify those that might function well in the aqueous environment of a washing machine.

6.3 Design Concept

We identified three different strategies described in detail below for our partners to consider as ways to use NADES to clean oily soils from laundry at low temperatures.

6.3.1 NADES as Co-solvents

We first propose the application of certain NADES as an addition to laundry detergent, simply as another solvent in the formulation. Solvents aid in breaking up soils and help make them more soluble (Durkee, 2014). Because of their low temperature and solubility properties, NADES could increase detergent effectiveness at removing oily soils at low temperatures. Several chemicals already known to form NADES may be interesting to consider for use as co-solvents (Figure 2). NADES are composed of at least one chemical that acts as a hydrogen bond donor and one chemical that acts as a hydrogen bond acceptor since the composite solvent results from the formation of hydrogen bonds (Durand, Lecomte, & Villeneuve, 2015). An example of a hydrogen bond donor already used extensively in NADES is glycerol, which Method currently uses as a solvent in its 4x laundry detergent. Testing glycerol as a NADES component may therefore be a feasible option for our partners.

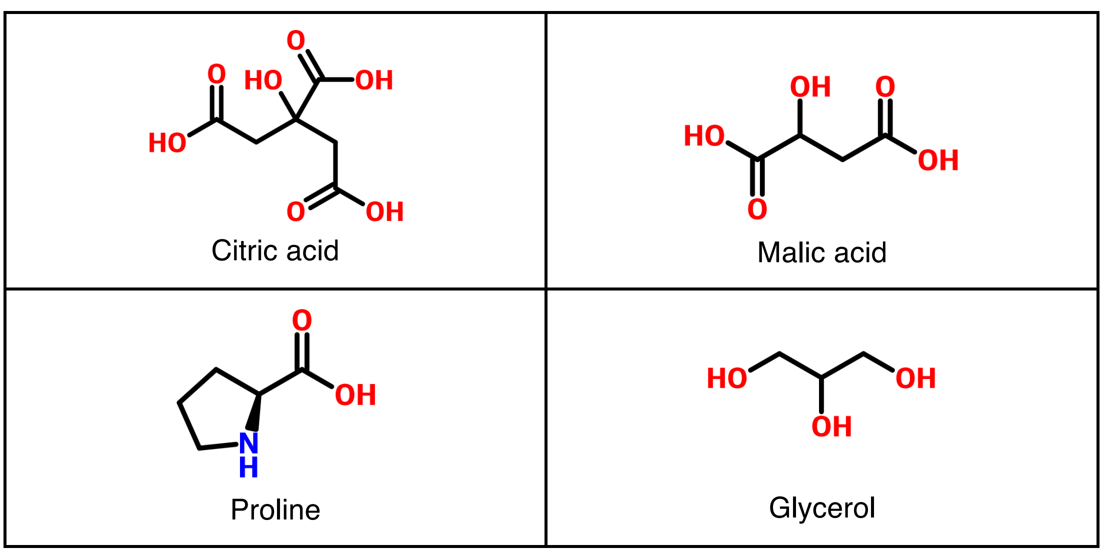


Figure 2. Chemicals already known to form NADES.

Indeed, all four chemicals shown in Figure 2 are likely to act as hydrogen bond donors, and have been paired with hydrogen bond acceptors such as choline chloride to make various NADES. However, since choline chloride is a quaternary ammonium salt it may present some toxic hazards if added to laundry detergent. We therefore propose considering NADES components that have not been previously assessed (Figure 3), which may potentially act as hydrogen bond acceptors. Succinic acid in particular may be promising; it also presents an opportunity for collaboration between our partners since BioAmber sustainably produces succinic acid and related derivatives.

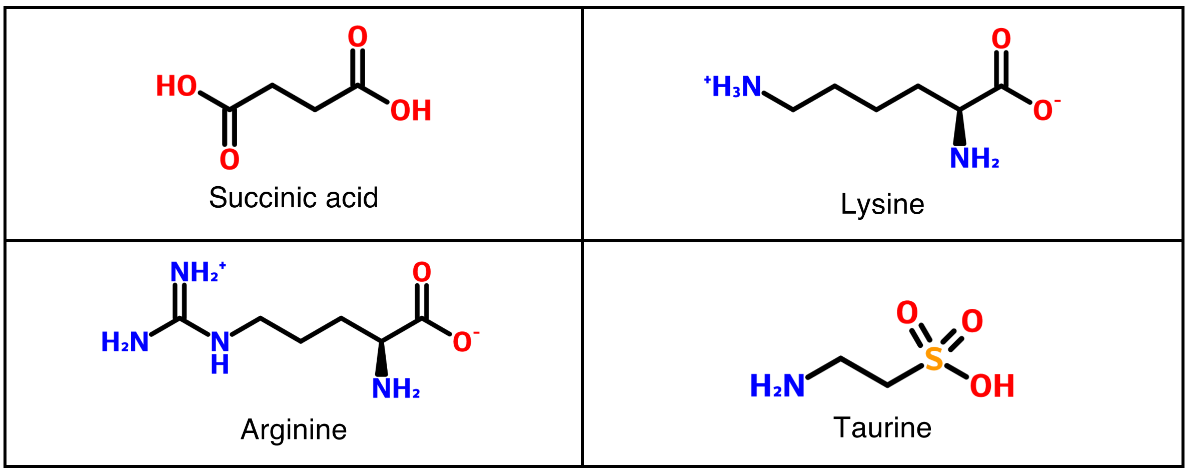


Figure 3. Potential NADES components that have yet to be experimentally tested.

6.3.2 NADES as Surfactants

Since NADES constitute relatively small molecules, their individual components may be useful as components of a surfactant in detergent formulation. Surfactants are especially important in detergent formulation for removal of lipophilic compounds from substrates. It may be possible to have certain NADES components undergo esterification reactions with alcohols that have long carbon chains to create amphiphilic surfactant-like NADES components (Figure 4). This proposal is purely hypothetical so we cannot know whether these compounds would indeed act as surfactants, or how they might interact with other detergent ingredients. However, given the substantial changes in chemical properties that NADES undergo when their components are combined, we think this might be an interesting concept to pursue further.

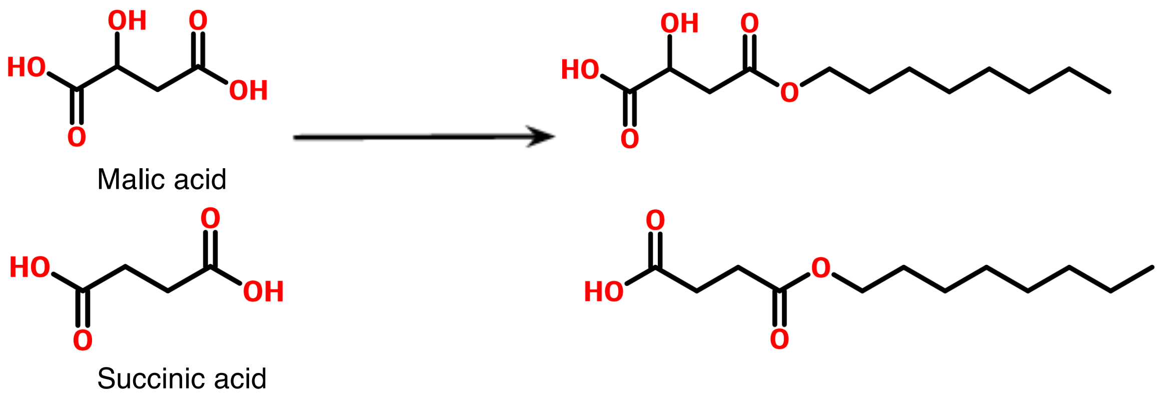


Figure 4. Potential amphiphilic NADES components synthesized by esterification with C_8 - C_{10} alcohols.

6.3.3 NADES as a Pre-treatment

NADES in a highly concentrated form may be particularly effective in a pre-treatment formulation. This would be applied to stained fabric or laundry before addition to the laundry machine. Given that high volumes of water may potentially decrease the ability of NADES to make oily particulates soluble or break apart the NADES completely, a pre-treatment application could allow NADES to function in a non-aqueous environment. NADES could potentially lose solvent functionality once the laundry machine reaches a critical water volume, but the individual chemicals may then act as dispersants, keeping soils broken up in solution and preventing redeposition onto the fabric substrate.

6.4 Human and Environmental Hazard Assessment

Human and environmental hazards of NADES are highly dependent upon what chemical components make up the chemical mixture. We expect that many of the NADES we propose for laundry application will pose low risks to human health. Not only are many of these chemicals abundantly found in nature, but they are also products of cellular metabolism and naturally occur in humans. These chemicals' toxicity may change, though, when they become incorporated into NADES.

A few studies have experimentally researched the potential toxicity of NADES. Hayyan et al. evaluated both *in vitro* and *in vivo* toxicity of four different NADES and concluded they may have toxic properties since they were shown to inhibit cancer cell growth at certain dosages (Hayyan, Looi, Hayyan, Wong, & Hashim, 2015). However, this study evaluated NADES as potential therapeutic agents—e.g. alternative drug delivery vehicles—and therefore used higher standards of safety than would be relevant to NADES use in laundry detergent. We would expect both the human exposure potential and the dose to be much lower than what was evaluated by Hayyan et al. for NADES application either in laundry detergent or as a pre-treatment.

Both Wen et al. and Radošević et al. evaluated the toxicity of cholinium-based solvents on various cell lines and found conflicting degrees of cytotoxicity and phytotoxicity (Radošević et al., 2015; Wen et al., 2015). It is clear that more experimental research should elucidate the toxic effects of NADES, particularly for chemicals other than choline salts. One interesting study demonstrated that NADES toxicity was lower than that of its component chemicals in aqueous solutions (Wen et al., 2015). The mechanism behind this observation is unknown, but it suggests that NADES formation may itself have an effect on the component chemicals' toxicity.

The biodegradability of NADES is also important to evaluate in order to understand what kind of environmental impact these materials will have once washed down the drain. Biodegradability is easily tested using a closed bottle test, which assesses how quickly theoretical oxygen levels are depleted when a compound of interest is added to an aqueous solution similar in composition to that of wastewater. A compound is considered "readily biodegradable" if there is at least 60% theoretical oxygen depletion within a 28-day period. Radošević et al. performed this test and found three different choline chloride based NADES were readily biodegradable (2015), while Wen et al. tested eight different NADES and found only two of them to be readily biodegradable (2015). Thus, biodegradability varies considerably across NADES.

It is clear from toxicity and biodegradability experimental research that properties of NADES are highly dependent upon their chemical make up. It will be important to conduct more research on the hazards of specific NADES if they are to be added to laundry detergent formulations.

6.5 Research Priorities

NADES may potentially increase the effectiveness of laundry detergents by acting upon oily stains at low temperatures when added as solvents to the formulation, when used to create surfactant like molecules, or when applied as a pre-treatment to fabrics. Since possible applications of these chemical mixtures have not been fully developed in the literature, more information needs to be obtained before considering NADES application in laundry. These research priorities include:

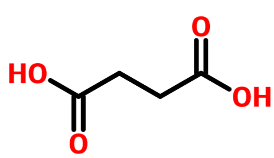
- Testing to better understand how water affects NADES solubilizing capabilities.
- An in-depth assessment of the toxicity and biodegradability for NADES of interest.
- Determining which NADES are most promising for laundry at low temperature.

7. Bio-based Solvents

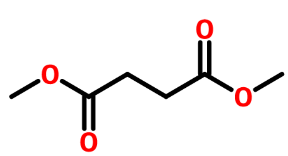
7.1 Inspiration

Our partner companies include innovators in chemical production technology and biotechnology. BioAmber produces a variety of compounds derived from succinic acid, which in turn is produced from renewable agricultural feedstocks. Dialkyl esters of succinic acid can potentially be used as solvents in cleaning formulations. BioAmber has already investigated their use as solvents for fragrances (Mullen, Bhat, & Thergaonkar, n.d.). Figure 5 shows the chemical structures of succinic acid and several dialkyl succinate esters. In this section, we use literature resources to investigate solvent properties and hazard traits of dialkyl succinates.

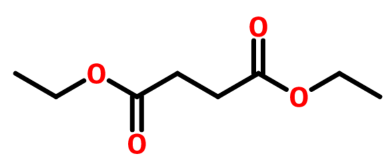
A variety of alkyl groups can be incorporated by esterification of succinic acid, leading to a family of solvents with a range of properties. BioAmber has produced esters with alkyl chain length ranging from methyl (C1) to dodecyl (C12). Based on consultation with Method and BioAmber, we selected a limited subset of this family of compounds, which includes representatives of three different groupings according to alkyl chain length, shown in Figure 5: 1) Short: Dimethyl succinate [DMSu] and Diethyl succinate [DESu]; 2) Medium: Bis(3-methylbutyl) succinate [D(3MB)Su], which is chemically representative of di-n-butyl succinate [DBSu] and di-n-amyl succinate [DASu]; and 3) Long: Dioctyl succinate [DOSu].



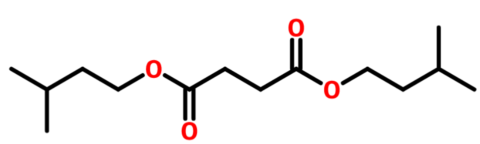
Succinic acid



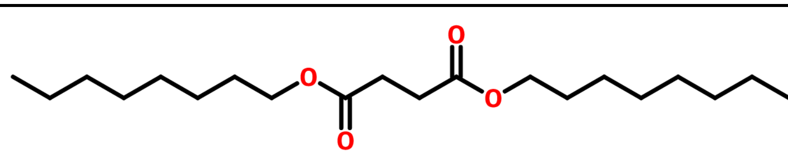
Dimethyl succinate [DMSu]



Diethyl succinate [DESu]



Bis(3-methylbutyl) succinate [D(3MB)Su]



Dioctyl succinate [DOSu]

Figure 5. Structures of selected succinic acid derivatives for consideration as solvents.

7.2. Technical Feasibility

One way to prospectively evaluate the effectiveness of new compounds as solvents is to use Hansen solubility parameters (HSP). HSPs are widely used in formulated product industries to quantify the functional properties of solvents (Hansen, 2004). HSPs form a three-dimensional solvent scale, in which the three parameters represent an empirical separation of chemical cohesive energy into three types of intermolecular forces: dispersion forces (dominant for highly non-polar compounds), polar forces, and hydrogen bonding.

Published HSPs are available for dimethyl succinate (Hansen, 2007). For the other compounds of interest, we computed HSP estimates using two different group contribution methods. These methods assign partial quantitative contributions to defined molecular fragments for each solubility parameter. By identifying all relevant fragments in the molecule and combining their contributions in a specified way, one can estimate the overall HSP for the molecule. Our calculation results, as well as the one available published set of HSPs, are shown in Table 2**.** The full calculations are included in Appendix 3.

Table 2**:** Estimated and published HSPs for several dialkyl succinate esters

HSP [MPa^{½}]	Estimated (Hansen 2007 method)			Estimated (Stefanis & Panayiotou 2008 method)			Published (Hansen 2007)		
Compound	**δ[D]**	**δ[P]**	**δ[H]**	**δ[D]**	**δ[P]**	**δ[H]**	**δ[D]**	**δ[P]**	**δ[H]**
Dimethyl succinate [DMSu]	11.9	5.1	8.8	16.0	11.4	8.9	16.2	4.7	8.4
Diethyl succinate [DESu]	13.2	4.1	7.9	15.9	10.7	8.1			
Dibutyl succinate [DBSu]	14.5	3.0	6.7	15.8	9.5	6.4			
Diamyl succinate [DASu]	14.9	2.6	6.3	15.7	8.9	5.6			
Bis(3-methylbutyl) succinate [D(3MB)Su]	12.9	2.9	6.7	15.3	8.2	5.9			
Dioctyl succinate [DOSu]	15.7	1.9	5.4	15.6	7.1	3.2			

Table 3: Known HSP values for some comparable solvents and relevant soils.

Substance	HSP [$MPa^{½}$]		
solvents (from Hansen, 2007)	**δ[D]**	**δ[P]**	**δ[H]**
ethyl acetate	15.8	5.3	7.2
isobutyl acetate	15.1	3.7	6.3
cetyl alcohol	15.1	3.7	8.1
methyl isoamyl ketone	16.0	5.7	4.1
soils (from Durkee, 2014)	**δ[D]**	**δ[P]**	**δ[H]**
fatty acid esters (olive oil)	15.9	1.2	5.4
unsaturated/saturated fatty acids (cottonseed oil)	12.2	5.8	5.8
saturated fatty acids (lard)	17.7	2.7	4.7
carbonized residue (coal tar pitch)	18.7	7.5	8.9
paraffinic oils (mineral oil)	15.7	0	0

Our estimates of HSPs for DMSu are roughly consistent with published values (which may themselves be somewhat approximate). The method of Stefanis and Panayiotou (2008) gave consistently higher polar parameter values than the classical method described in Hansen (2007). Comparing the estimated HSP values for dialkyl succinates against those of some common solvents and soils (Table 3) shows that these solvents are comparable to conventional alkyl esters, ketones, or even fatty alcohols. These solubility parameters suggest potential effectiveness on soils such as vegetable oils, fats, and burnt organic material. Empirical testing is the best way to evaluate the actual effectiveness of these solvents in real applications.

7.3 Design Concept

We propose that bio-based dialkyl succinates can serve as either additional ingredients or as direct replacements of current solvent ingredients. We predict they will boost the low-temperature effectiveness of laundry formulations with lower human health risk and environmental impact due to sourcing from renewable feedstock.

7.4 Human and Environmental Hazard Assessment

7.4.1 Physical Hazards and Exposure Potential

Dialkyl succinates can be expected to have low or moderate flammability, based on computationally estimated flash points (computed with the ACD/Percepta platform, available in ChemSpider). Only DMSu, with an estimated flash point of 85 °C, would be classified by GHS as a combustible liquid (Category 4; GreenScreen: moderate flammability). None of the medium- and long-chain esters would be classified for flammability in GHS (GreenScreen: low flammability).

These solvents are generally semi-volatile, indicating a low exposure potential during manufacturing and use. With boiling points ranging from 196 °C (DMSu) and 216°C (DESu), the short-chain esters fall just barely within the US EPA's technical classification of volatile organic compounds (VOCs), while the medium- and long-chain esters fall within the semivolatile organic compound (SVOC) designation (US EPA, 2015). Vapor pressures of DMSu (0.1 mmHg) and all longer-chain solvents are very low.

Another consequence of low volatility, however, is that any solvent residues remaining on washed clothes may fail to completely evaporate. This could lead to repeated low-dose exposures to consumers, especially those who add too much product to the washing machine.

7.4.2 Human Health Hazards

We found few health and safety studies on these compounds and therefore cannot evaluate in detail their human health effects. Most existing studies concern DMSu. Acute inhalation of DMSu vapor or aerosol at very high concentrations (5.9 g/m^3) causes damage to nasal tissue in rats (Lee, Valentine, & Bogdanffy, 1992), with the monomethyl ester and succinic acid as potential toxic metabolites (Trela & Bogdanffy, 1991). If succinic acid and its monoesters are indeed toxic metabolites in mammals, then diethyl and other esters may be expected to exhibit similar toxicity. However, such extreme inhalation exposure is highly unlikely in the application of this semi-volatile substance as a minor component of laundry formulation—with the possible exception of occupational exposures due to catastrophic releases. Furthermore, the toxicity is likely to be specific to the inhalation exposure route, since DMSu is used as a food additive to solubilize artificial fruit flavors (US National Library of Medicine, 1997).

Receptor binding scores calculated using LASSO (via ChemSpider) for DOSu, DASu, and D3MBSu predicted very low affinities to the range of receptors modeled. The maximum score was 0.03 for both ACE (angiotensin-converting enzyme) and PPARs (a type of nuclear hormone receptor). This simply suggests an absence of immediate warnings for human health hazard.

7.4.3 Environmental Hazards

EPI Suite (US Environmental Protection Agency, 2012a) provides computational estimates of key environmental fate, partitioning, persistence, and bioaccumulation properties of several dialkyl succinates. EPI Suite predicts ready biodegradability (GreenScreen: *very low* persistence) for all succinate diesters that we examined. The primary ester groups and linear aliphatic chains featured in these molecules are associated with fast environmental and biological degradation. Based on estimated physicochemical properties, EPI Suite predicts that short and medium alkyl esters would partition predominantly into water and soil, while DOSu would partition predominantly to soil and sediment. The model predicts relatively high persistence for these compounds in sediment (78 - 135 days; GreenScreen *high* persistence) and overall persistence times ranging from 17 to 28 days. While these model predictions are our only current source of information (in the absence of empirical data), they are not fully reliable and they are somewhat self-contradictory. We do not consider persistence to be of concern for these compounds, but ultimately, experimentally studying their environmental fate is a research priority.

Environmental and metabolic degradation of these compounds is likely to proceed through hydrolysis and oxidation. Hydrolysis would yield monoalkyl esters, the corresponding alcohols, and succinic acid. Virtually no toxicological data is available on monoalkyl esters of succinic acid. Succinic acid itself is a normal cellular metabolite involved in the Krebs cycle. It is a weak acid ($pK_1 = 4.2$, $pK_2 = 5.6$), and we expect it to be rapidly degraded and benign in the environment. Likewise, the alcohols ranging from methanol to octanol are expected to be rapidly degradable.

Predicted bioconcentration factors (BCF) for all five solvents above correspond to *low* (D3MBSu) or *very low* bioaccumulation potential according to GreenScreen criteria, with the only exception being DOSu. With a predicted BCF of 844 L/kg, and predicted $K_{ow} \approx 7$, $K_{oa} \approx 10$, the estimated physicochemical properties of DOSu fit the profile for potential aquatic and terrestrial bioaccumulation (Howard & Muir, 2010), and would correspond to *moderate* bioaccumulation potential in GreenScreen. However, DOSu could be readily degraded by hydrolysis, oxidation, or other cellular metabolic pathways. Therefore, the true BCF and overall bioaccumulation potential of DOSu is likely to be lower than what is predicted based on its physical properties alone. We expect the other dialkyl succinates to be rapidly metabolized as well, although D(3MB)Su has branched aliphatic groups that may hinder certain cellular metabolic pathways. As with persistence, further study of bioaccumulation potential is needed to rigorously evaluate the safety of these compounds.

7.5 Research Priorities

To implement this strategy, we recommend that our partners conduct empirical testing or further research on the following factors:

- The effectiveness of dialkyl succinate solvents, including combinations of solvents, in dissolving soils of interest.
- Solvent compatibility with existing laundry formulations and effectiveness in those formulations at low temperatures.
- Testing to close the remaining data gaps concerning toxicological and ecotoxicological properties.

8. Biosurfactants

8.1 Inspiration

Many bacteria and fungi, such as *Pseudomonas Aeruginosa* and *Cladosporium resinae,* secrete surface-active compounds that function as natural surfactants, or biosurfactants, by reducing surface and interfacial tension between two media. In addition, they form micelles and microemulsions that support oil particle dispersion in aqueous environments (Banat et al., 2010). These multi-purpose biosurfactants support biological organisms in a wide variety of functions, including digesting hydrocarbons or oils for nutrient intake and attaching to hydrocarbon substrates to facilitate growth in myriad environments (Davey, Caiazza, & O'Toole, 2003; Ron & Rosenberg, 2001). Indeed, biosurfactants help increase surface area at the oil to water interface, allowing more bacteria to feed on oil droplets. They further allow the bacteria to detach from the substrate once the food source has been consumed (Ron & Rosenberg, 2001). Some bacteria also secrete biosurfactants that assist in organizing larger microbial communities called biofilms by regulating open channels or pores that facilitate hydration, nutrient dispersion, and waste disposal for the entire microbial community (Davey et al., 2003). On a larger scale, biofilms aid in marine petroleum oil spill bioremediation (Yakimov, Timmis, & Golyshin, 2007). This ability to promote cell-to-cell and cell-to-surface interactions at various interfaces position biosurfactants in a unique role to potentially aid in removing oily stains from clothing in a water-filled laundry machine.

8.2 Technical Feasibility

8.2.1 Biosurfactant Structure and Function

Glycolipids are one class of low molecular weight biosurfactants that are formed from carbohydrates in combination with long-chain aliphatic acids or hydroxyaliphatic acids. They include sophorolipids, rhamnolipids, trehalolipids, and mannosylerythritol lipids (MELs) (Banat et al., 2010). Sophorolipids and rhamnolipids are currently considered the most promising for detergent use (Müller et al., 2012). Sophorolipids are more established in current products than rhamnolipids (de Guzman, 2015b; Müller et al., 2012), but there has been growing interest and effort to develop both new rhamnolipids and sophorolipids for various commercial uses (de Guzman, 2015a; Delbeke, Movsisyan, Van Geem, & Stevens, 2016; McKeag, 2015; Müller et al., 2012).

Rhamnolipids are generally synthesized from bacteria and are made up of a hydrophobic tail of fatty acids or lipids joined to one or two naturally occurring hydrophilic deoxy sugar molecules (i.e. Rhamnose) (McKeag, 2015). Sophorolipids are generally synthesized from yeast or fungi and form a hydrophobic fatty acid tail with a hydrophilic carbohydrate head from a glucose disaccharide (i.e. Sophorose) (Davila, Marchal, & Vandecasteele, 1994). Figure 6 shows the structure of Rhamnolipid 1, a biosurfactant secreted by *Pseudomonas Aeruginosa*.

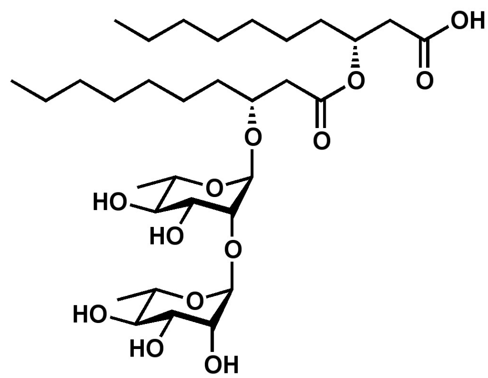


Figure 6. Rhamnolipid 1 (Boghog https://commons.wikimedia.org/wiki/File:Rhamnolipid.tif).

Figure 7 shows the lactonic sophorolipid (a) and acidic sophorolipid (b) structures, both of which are secreted by the fungus, *Cladosporium resinae*. Some studies have found that biosurfactants like the closed-ring lactonic sophorolipid can form a variety of 3D structures in solution (Penfold et al., 2011). In addition to micelles, the closed-ring sophorolipids may also potentially form cylinders, sheets, and multi-layered micelles called lamellar vesicles (Ho, 2000; Penfold et al., 2011). Lamellar vesicles are currently used in some liquid laundry formulations, where they help to suspend insoluble ingredients, suggesting that biosurfactants that can self-assemble into 3D structures may be particularly useful for future laundry applications.

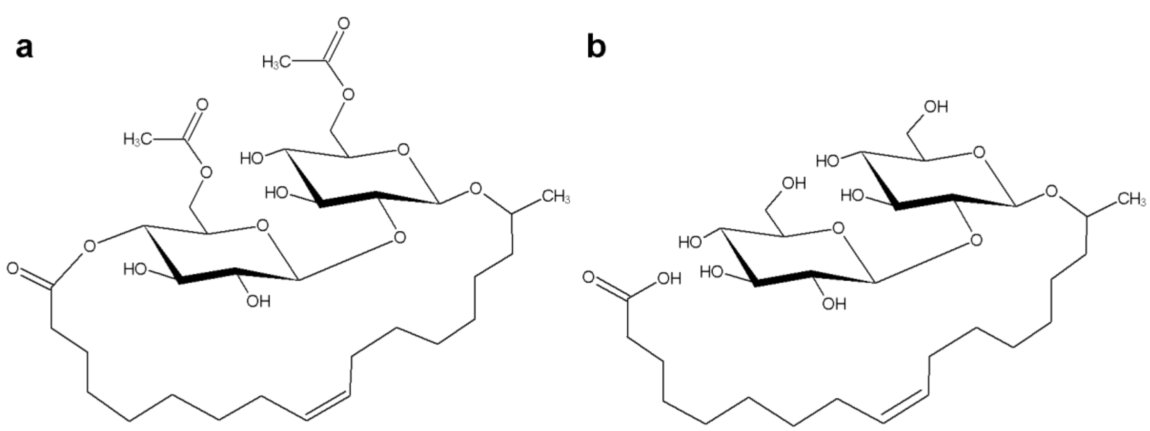


Figure 7. Two sophorolipid structures (a) lactonic (b) acidic (BioSurfing project, http://www.bbeu.org/biosurfing).

Another kind of surfactant found in nature is a cyclic lipopeptide called Surfactin (Figure 8), a natural antibiotic produced by *Bacillus subtilis* (Ron & Rosenberg, 2001). Surfactin is a powerful biosurfactant in terms of its ability to reduce surface tension (Desai & Banat, 1997; Ron & Rosenberg, 2001). One study also demonstrated Surfactin's ability to combine in an additive fashion with other laundry detergent components to effectively improve wash performance at low temperatures (Mukherjee, Das, & Sen, 2006). More research would be necessary to determine whether this potential additive property is widespread among biosurfactants, but it may be promising as a formulation-level approach for Surfactin and other biosurfactants.

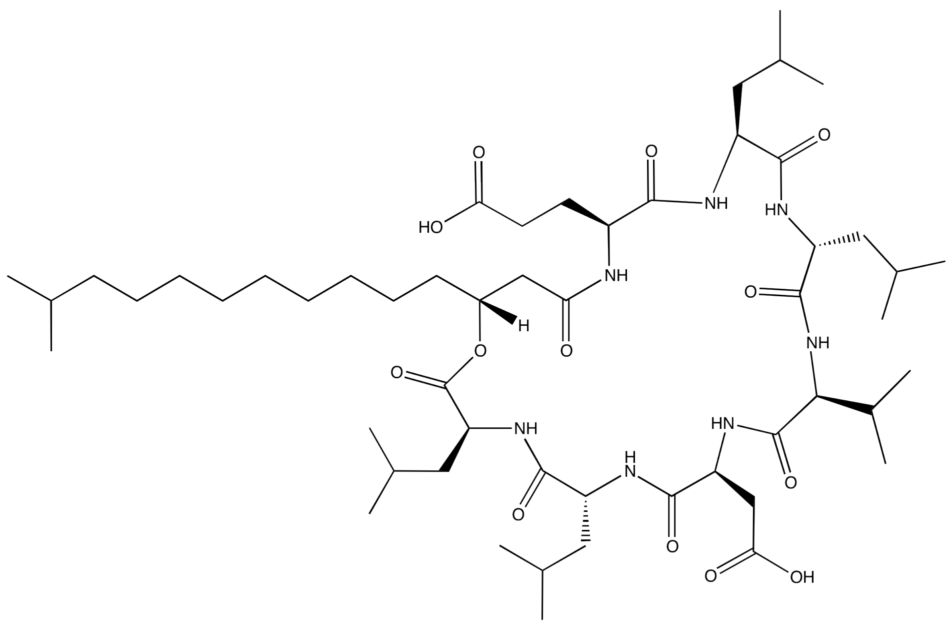


Figure 8. Surfactin structure.

8.2.2 Promising Surfactants From Renewable Resources

Biosurfactants were originally identified as promising specifically for oil recovery and bioremediation in the 1980s, but more recent attention has been given to their potential use in cleaning detergents and other consumer products (Delbeke et al., 2016; Mukherjee et al., 2006; Müller et al., 2012). As seen in Figure 9, the number of publications and citations for sophorolipids alone has increased dramatically in the last 10-15 years (Delbeke et al., 2016).

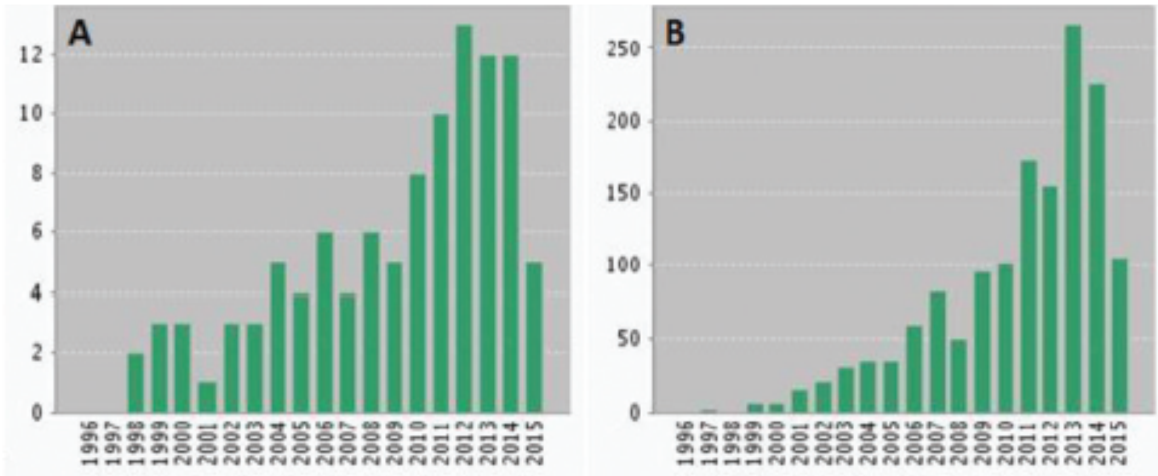


Figure 9. Number of publications (a) and citations (b) per year on sophorolipids.

Their performance-enhancing properties, such as wetting, dispersing, surface tension reduction, and ability to form 3D structures, in addition to their relatively low environmental and health impacts, have positioned biosurfactants as promising alternatives to non-renewable surfactants (Delbeke et al., 2016; Mukherjee et al., 2006; Müller et al., 2012). They are considered to be less toxic and more biodegradable than conventional surfactants (Delbeke et al., 2016; Hirata et al., 2009; Müller et al., 2012), and may additionally have antimicrobial and antifungal properties that enhance their value to a variety of high demand markets beyond cleaning (Mukherjee et al., 2006; Müller et al., 2012).

Even with this growing interest, the biggest challenge to large-scale biosurfactant commercialization is production costs associated with scaling up (Müller et al., 2012). However, there is a great deal of current work on how to make large-scale production more cost effective. A number of academic researchers and companies are developing novel ways to optimize large-scale production and/or are using biosurfactants in their products. Companies like Ecover, Evonik, and Saraya (a Japanese company) use sophorolipids in some of their dish and laundry detergents (de Guzman, 2015b; Delbeke et al., 2016). Logos Technologies has a line of rhamnolipid products, and GlycoSurf has developed a cheaper way to biosynthesize rhamnolipids inspired by research efforts of Jeanne Pemberton's research team at the University of Arizona (de Guzman, 2015a; McKeag, 2015). Many other chemical and enzymatic modifications are currently being explored for biosurfactant production optimization (Delbeke et al., 2016), indicating that cost-effective production, and thus commercialization, is within reach.

In addition to chemical biosynthesis, another way to produce biosurfactants is through laboratory fermentation. One of our partner companies, Amyris, produces a number of different chemical compounds using a yeast model that we think could potentially apply to biosurfactants (Figure 10). The Amyris yeast model breaks down sugar biomass into elemental building blocks (i.e. sugar syrup) that are then reassembled into various compounds like Farnesene using a genetically altered yeast metabolism pathway, called the Mevalonate Pathway. We propose this as an opportunity for future research to see whether this pathway could prove promising for biosurfactant production, particularly for sophorolipids since they are typically synthesized by yeast.

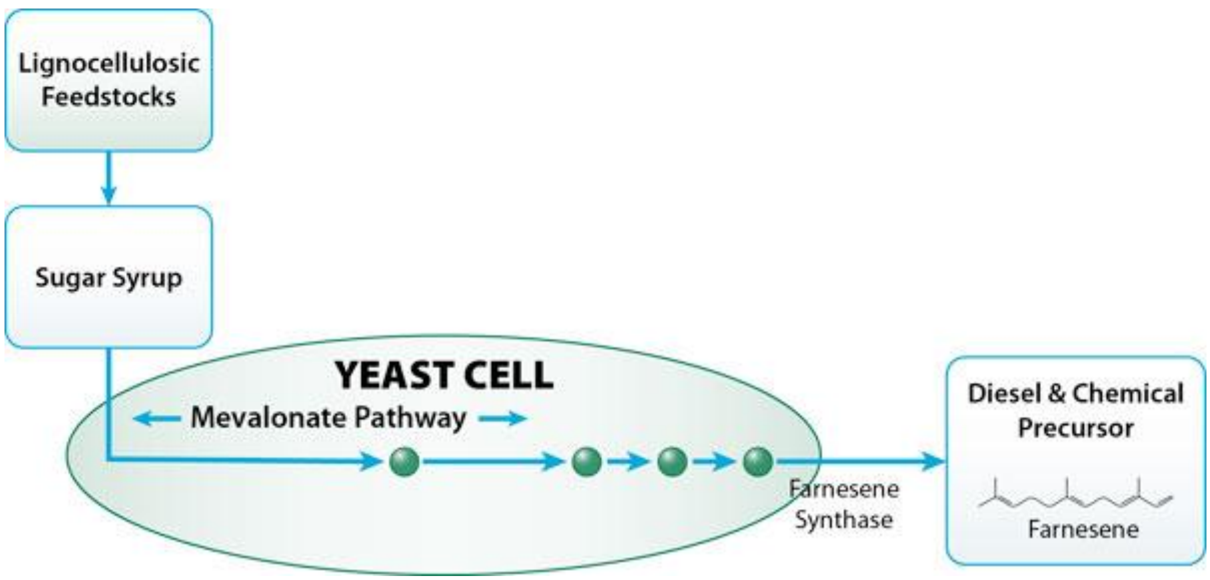


Figure 10. Simplified schematic of Amyris' yeast model (http://www.nabcprojects.org/images/amyris_graphic.jpg).

8.3 Design Concept

We propose direct replacement of one of the currently used surfactants in laundry detergent, which include sodium lauryl sulfate (SFS), lauryl ethoxylate, PEG 600 monooctyl ether, and soy methyl ester ethoxylate, with a novel bioinspired or bioengineered surfactant, such as a rhamnolipid, sophorolipid, or a cyclic lipopeptide like Surfactin. If Amyris technology could generate such a biosurfactant, we propose prioritization of sophorolipid production because it may be the most promising and realistic option. In addition to direct ingredient substitution, a formulation approach that explores additive properties of biosurfactants with other laundry detergent ingredients may also be appropriate, but more thorough research is needed to understand whether this is a viable opportunity.

8.4 Human and Environmental Hazard Assessment

Biosurfactants score fairly well on both environmental and human toxicity. They have been shown to readily biodegrade by standard OECD guidelines and to have low cytotoxicity to human tissue cells (Delbeke et al., 2016; Hirata et al., 2009). They have also been shown to have lower acute human toxicity in terms of skin and eye irritation than conventional surfactants, but natural surfactants do appear to exhibit mild aquatic toxicity (Delbeke et al., 2016). Existing data indicates that this aquatic toxicity is still somewhat lower than synthetic surfactants (Delbeke et al., 2016), but there are clearly toxicity data gaps that have yet to be addressed for biosurfactants.

8.5 Research Priorities

Biosurfactants can potentially serve to boost the low-temperature effectiveness of laundry formulations through direct ingredient substitution and possibly through formulation manipulations. To implement this strategy, we recommend that our partners conduct empirical testing or further research in the following areas:

- The feasibility of producing a 1) Sophorolipid, 2) Rhamnolipid, and/or 3) Cyclic lipopeptide through the Amyris yeast model.
- The effectiveness of 1) Sophorolipids, 2) Rhamnolipids, and 3) Cyclic lipopeptides in dissolving soils of interest.
- Biosurfactant compatibility with existing laundry formulations and effectiveness in those formulations at low temperatures.
- Potential additive properties with other laundry ingredients at low temperatures.

9. Enzymes

9.1 Inspiration

Enzymes are proteins present in all living cells. They exist in humans and other animals, plants, bacteria, fungi, and yeast (Hasan et al., 2013). Within cells, they perform the important function of controlling metabolic processes by converting nutrients into energy and new cell material (Vanhanen, 2001). Enzymes are catalysts that speed up chemical reactions without being consumed in the process (D. Basketter et al., 2012). In nature, enzymes degrade carbohydrates, proteins, and lipids by breaking bonds with great specificity; they can degrade or synthesize specific compounds or even target one particular type of bond (Vanhanen, 2001). Their specificity and efficiency make enzymes ideal instruments for industrial processes. They also can exist at a relatively wide range of temperatures and pHs since they are abundant in living systems (D. A. Basketter et al., 2008; Hasan et al., 2013).

Enzymes are an important part of laundry detergent because they are able to break down particularly complex hydrophobic compounds into smaller and more soluble fragments (Aehle, 2004). As of 1990, over half of all laundry detergent products in developed countries contained enzymes (Chaplin & Bucke, 1990). Protease, amylase, and mannanase are currently used in Method and Seventh Generation laundry and automatic dishwashing detergents. Protease breaks down proteins, amylase breaks down carbohydrates, and mannanase breaks down mannans, or common binding additives such as guar gum (Hasan et al., 2013). In laundry applications, these enzymes can target the breakdown of various chemical classes and facilitate the removal of stubborn complex oily soils. Lipase is a type of enzyme that breaks down lipids and is currently used in some laundry applications and recommended for pre-soak oil removal (Hasan et al., 2013). Lipase hydrolyzes triglycerides into glycol and free fatty acids (Figure 11). It is also effective at low temperatures and alkaline pHs (Chauhan, Chauhan, & Garlapati, 2013; Hasan et al., 2013). However, there are several technical constraints for lipase use in laundry detergents, which are discussed in detail below.

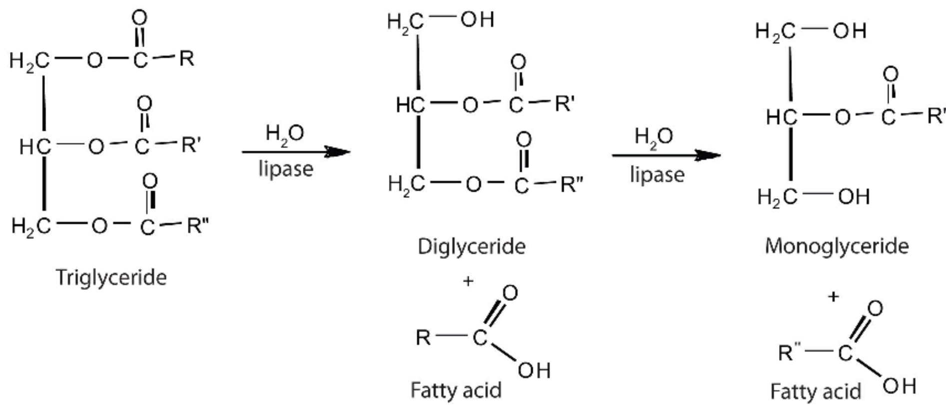


Figure 11: Hydrolysis of triglycerides by lipase into glycerides and free fatty acids.

9.2 Technical Feasibility

Lipase breaks down triglycerides by breaking off their hydrocarbon chains, making them more hydrophilic. Thus, the glyceride and free fatty acid chain products become more soluble, allowing for more effective removal. The limitation of lipase entails the production of butyric acid, a byproduct of the triglyceride hydrolysis, which produces an unpleasant odor. This malodor is of particular concern during the breakdown of pure lipid soils such as lard or grease.

Studies have shown effective lipase activity over three washing cycles and into the drying cycle, a relatively long activity time that contributes to the continued production of a malodor. Lipase activity is also sustained during the drying cycle due to optimum conditions of lipase in 20-30% water content (Chauhan et al., 2013). We estimate that malodor is a greater concern during the drying cycle than the wash cycle; butyric acid formed in the washing cycle may be washed away, while continued malodor production in the drying cycle is likely to persist on dry clothes.

Stabilizing ingredients such as diols, calcium chloride, citric acid, or boric acid are needed to preserve enzymes prior to consumer use if used in liquid detergent products. This is an additional step required for formulation, however, not a limiting factor.

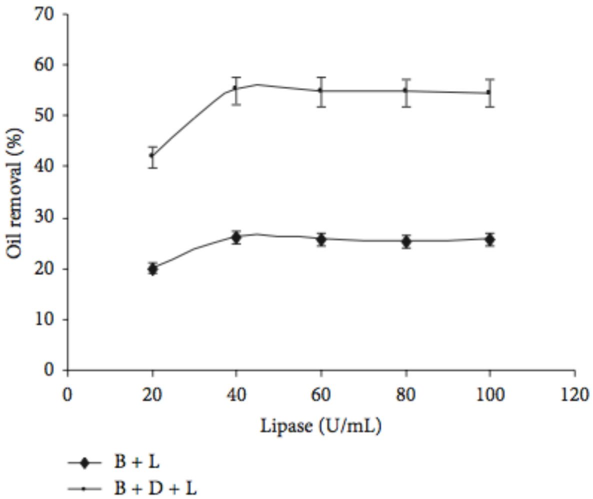
9.3 Design Concept

We propose to use lipase directly in laundry detergent or as a pre-treatment before the wash cycle begins. Several newly developed lipase strains show promise in their low temperature performance, length of activity, and interaction with other ingredients.

Alkaline yeast lipases, or cold-active lipases, may be preferred to fungal and bacterial lipases due to their enhanced ability to work at lower temperatures (Hasan et al., 2013). Some bacterial lipases can also operate well at low temperatures. Although optimal activity for two lipases produced by Staphylococcus sp. strain ESW and *Bacillus stearothermophilus* was observed at 50-60 degrees Celsius, residual activity was observed at 30 degrees as well, indicating continued performance in laundry at low temperatures (Cherif, Mnif, Hadrich, Abdelkafi, & Sayadi, 2011). Both lipases are very active in alkaline conditions, highly stable with nonionic and anionic surfactants, and relatively stable in the presence of oxidizing agents (Cherif et al., 2011).

Some lipases also work additively in combination with other compounds to remove tough stains. A new lipase produced by an engineered strain of *Staphylococcus arlettae (*JPBW-1) provides optimal oil removal in combination with nonionic surfactants (Figures 12 and 13)(Chauhan et al., 2013). Researchers observed maximum oil removal activity at 37 degrees C, which is too hot to be considered low temperature, but they also noted additive activity as low as 25 degrees C. Authors of this study also concluded that lipase would be an effective ingredient in a "pre-soak" solution in combination with a nonionic surfactant, which may allow for optimum water content as well as a longer contact time with lipids to reduce or prevent malodor development during the drying cycle (Chauhan et al., 2013).

Thus, lipase may prove to be promising for pre-treatment, especially if used in combination with a nonionic surfactant. Lipase may also interact with other ingredients at the formulation level. Some surfactants have been shown to strongly inhibit lipase activity (Aehle, 2004), which could effectively reduce the lengthy activity of lipase and thus optimize its utility in laundry detergent formulations. Lipase has also been shown to work synergistically in laundry applications with other enzymes like protease (Jiang, Yin, & Ren, 2004). The ability to interact additively, synergistically, and/or antagonistically with different compounds in various conditions makes lipase an interesting candidate for further study in removing oily stains at low temperatures at both the formulation and process levels.



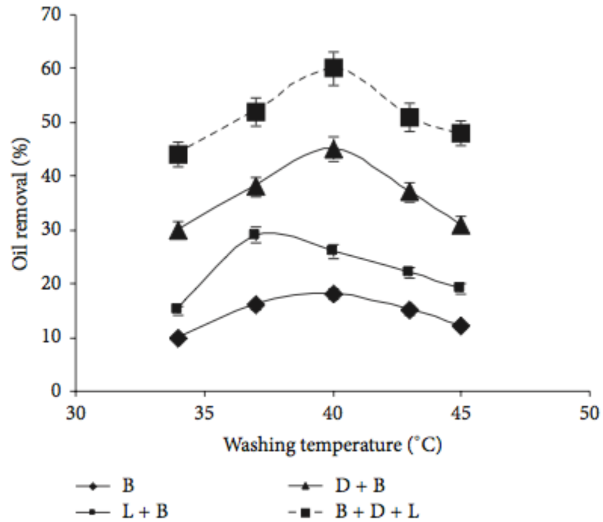


Figure 12 (left): Additive interaction of lipase produced from S. arlettae and nonionic detergent for approximately 62% oil removal.

Figure 13 (right): Washing temperature of 40 degrees C demonstrated optimal oil removal for nonionic surfactant-based detergent and lipase solution.

9.4 Human and Environmental Hazard Assessment

Enzymes are ubiquitous in living systems, which indicates they are relatively safe compounds for human health and the environment. As proteins, they are readily degradable in the environment (Vanhanen, 2001). In addition to being biodegradable, they are also non-toxic and leave no harmful residues (Hasan et al., 2013). However, enzymes do pose a human health risk of irritation and sensitization if exposure doses are large and if the duration of the exposure is chronic.

Protease was first added to laundry detergent in 1959 (Vanhanen, 2001). In the late 1960's and early 1970's, protease in laundry detergent was linked with respiratory irritation, sensitization, and allergies from occupational exposure (Vanhanen, 2001). There were also some cases of dermal irritation, although the majority was respiratory-related. Employees were exposed to "dusty" working conditions with relatively high doses and chronic exposure to respirable particles. Exposure has since been controlled with formulation changes and encapsulation of the enzyme granules, as well as improved industrial hygiene practices (Hasan et al., 2013; Vanhanen, 2001). However, diligent industrial hygiene measures such as proper ventilation, enclosed enzyme use, and personal protective equipment are still very important to protect workers who are occupationally exposed to enzymes. A case-control study performed in 2000 revealed a

22% excess risk of sensitization attributable to enzyme exposure (Vanhanen et al., 2000).

On the other hand, enzymes pose a low health risk for consumers due to low exposure dose and short exposure duration. Sarlo et al. conducted exposure assessments of normal consumer use of laundry detergents containing enzymes and measured extremely low airborne enzyme levels, indicating low exposure potential (Sarlo et al., 2010). Proteolytic enzymes such as protease are shown to have the highest potential of irritation and sensitization compared to other classes of enzymes. Given that protease is currently used in Method and Seventh Generation laundry detergents, the addition of lipase would realistically produce no excess health risk to the consumer. There is currently no evidence of developmental toxicity, reproductive toxicity, or carcinogenicity of enzymes.

There is currently no evidence of aquatic or environmental toxicity of enzymes, with a water partition coefficient (K_{oc}) of <1.3 (D. Basketter et al., 2012). In the instance that enzymes would survive in wastewater, they would likely be denatured in the wastewater treatment process. In the unlikely but possible instance that detergent-containing enzymes would be discharged directly into a waterway, they are estimated to biodegrade readily, with no threat of persistence.

9.5 Research Priorities

Mass production of microbial lipases has increased in recent years due to the attractive uses of lipase in a variety of industries including dairy, food, detergents, textile, pharmaceutical, cosmetic, and biodiesel; an estimated $7 billion dollar industry in 2013 (Hasan et al., 2013). Research is currently investigating the increased performance of cold-active lipases as well as novel and highly efficient lipase production (Cherif et al., 2011; Joseph, Ramteke, & Thomas, 2008). It is recommended that lipase be tested in laundry detergent formulation and as a pre-treatment solution in order to:

- Evaluate its effectiveness in conjunction with other compounds, including other enzymes, surfactants, biosurfactants, and NADES.
- Determine a formulation or pre-treatment cleaning process that would limit the production of butyric acid.

10. Oil-adhesive surfaces

10.1 Inspiration

The leaves of the sacred lotus (*Nelumbo nucifera*) have a top surface that repels water (superhydrophobic) and a bottom surface that resists oily soils (superoleophobic). The chemical composition of the wax that coats these leaves does not explain these remarkable properties. Instead, it's the nanoscale physical form of the surface—its particular 'roughness'—that prevents liquids from wetting the leaf. A number of chemical designs have attempted to produce this phenomenon synthetically. Chemists have succeeded at producing superhydrophobic and superoleophobic surfaces using nanotechnology, polymers, and fluorinated compounds (Brown & Bhushan, 2016).

We, in turn, were inspired to ask what potential cleaning applications could be imagined for technologies that enable such powerful discrimination between oils and waters at solid-liquid interfaces. The ability to easily separate oil from water could potentially lead to an innovative cleaning process, if 'oil' refers to suspended oily soils in the water/detergent mixture. We surveyed a range of new, experimental scientific literature to develop a highly speculative strategy. We propose a physical device that can be added to laundry to absorb (or rather, *adsorb*) oils that have been lifted out of fabrics and are suspended in the wash water. This device could help 'trap' soils and prevent their redeposition onto clothes as the water drains.

10.2 Technical Feasibility

Oil-water separation at surfaces is a relatively new technological niche. Still, a range of experimental technologies exists. Most of them are unlikely to be practical or environmentally preferable, and here we review a small selection that informs our proposed strategy.

10.2.1 Phase Separation by Filtering

Researchers have created a nanostructured surface treatment technology based on an unusual acrylate polymer (He et al., 2015). The polymer is polyzwitterionic—it contains multiple functional groups that each have both positive and negative charges in close proximity. It is based on phosphorylcholine, a naturally-occurring zwitterion, and is therefore claimed to be biomimetic. It is extremely hydrophilic and very good at repelling oil -even able to remove oil from itself with just water-and thus can be considered self-cleaning.

This coating can be used to make a filter-like device (by surface-treating a simple steel mesh), which allows water to pass through while blocking the flow of oils, thereby effecting oil-water separation. While intriguing for a variety of reasons (such as oil spill remediation, as the authors suggest), we could not imagine a compelling design implementation of such a filter in laundry washing. The bulk flow of liquid into and out of a laundry machine does not offer the same opportunities for 'filtering', since clothes themselves are not part of the water phase and would necessarily be left behind together with the oil phase.

A more desirable solution would produce the inverse effect: allowing oily soils to be separated away, leaving water and everything else behind. We identified one particular new technology that enables something that comes close to this.

10.2.2 Selectively Trapping Oil

In some very recent work, Uttam Manna, David Lynn, and co-workers described technology for creating multi-layered surface coatings with nanoscale pore structures (Broderick, Manna, & Lynn, 2012). These coating materials are polymeric and based on polyethyleneimine. They have further devised a way to functionalize this surface coating, meaning the chemical character of its surface can be modified independently of its underlying physical form. The result is a range of surfaces that can be "tuned" for varying degrees of hydrophilicity/hydrophobicity and also oleophobicity (Manna & Lynn, 2015).

The critical finding, from our perspective, is that some varieties of this surface treatment technology confer varying degrees of oil adhesiveness, while remaining superoleophobic. This means that the surface does not get soiled or 'wetted' by oils, but oils still adsorb or 'stick' to the surface in droplets. Importantly, this property also works underwater.

Hypothetically, an oil-adhesive superoleophobic surface could be engineered to collect, separate, and sequester oil by adsorption from an oil/water mixture. If this is effective, it could be used to prevent the redeposition of oily soils in laundry. However, while researchers have empirically demonstrated oil-adhesiveness, they have not conducted a larger-scale test of the technology in this way. Instead, Manna and Lynn conducted a different test, constructing a phase-separating filter apparatus (similar in concept to that of He et al., 2015), which takes advantage of a differently-tuned surface that is superoleophobic and hydrophilic but not oil adhesive.

For the following discussion, we will presume that this oil-adhesive surface technology could be made to function under laundry washing conditions.

10.3 Design concept

We propose a durable, reusable object that is liquid-permeable and has high internal surface area (Figure 14). It has a superoleophobic oil-adhesive coating on the interior surfaces. As oily soils are broken up and suspended in the wash water, they pass through this device and stick to its inner surfaces (but not outer, which would rub against clothes and redeposit soils). After the wash is complete, the device could be picked up by hand and re-used until it accumulates too much dirt to be effective. At that point it would require regenerating the surface by degreasing or cleaning off the soil in a safe and effective manner.

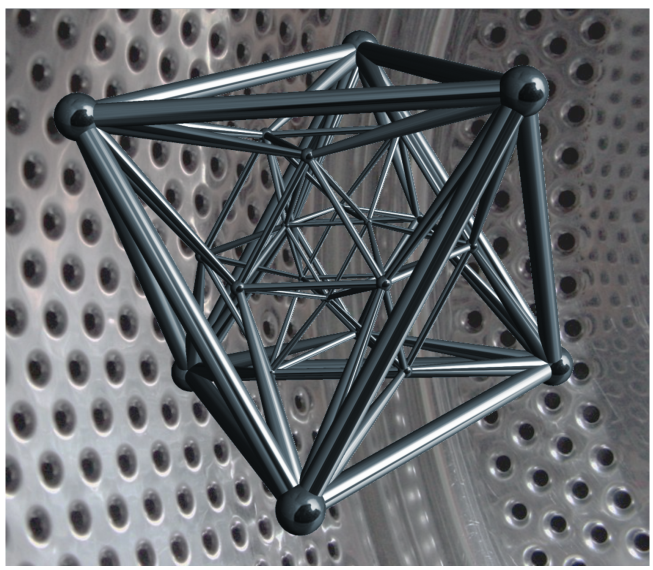


Figure 14. Proposed oil-trapping washing machine device (image by Andrew Kelsall. https://www.flickr.com/photos/andrewkelsall/4188019817/).

10.4 Human and Environmental Hazard Assessment

Several environmental and human toxicity concerns need to be addressed before implementation of this design concept. For one, the ethyleneimine-based polymer coating developed by Lynn and colleagues is derived from aziridine—a carcinogen, mutagen, and acutely toxic substance. This is an inherent drawback of the technology itself, and there are as of yet no substitutes that we can identify. Second, the need for eventual cleaning of oily soils off this device's chemically treated surfaces raises another challenge, which is in essence a secondary version of the fundamental challenge of removing oily soils from substrates (i.e., clothing). There may be new exposure concerns associated with this secondary cleaning. If, for example, the surface requires solvent degreasing, then consumers may engage in unsafe direct use of solvents to accomplish that task. Lastly, there are complex environmental and social impacts associated with manufacturing durable objects for mass consumption. The most obvious problems are waste, the product's end-of-life fate (especially if it can only be used a few times), and occupational hazards of chemical-intensive manufacturing.

Due to the niche and experimental nature of this technology we have no solutions to the problem of aziridine-based chemicals. But as to the problems of reusability and product life-cycle management, we suggest two approaches that can work individually or in tandem to reduce environmental health and sustainability concerns. The first approach enlists a cradle-to-cradle design. According to this paradigm, the product should either be infinitely recyclable, feeding its materials back into the manufacturing process as a "technical nutrient"; or biodegradable and safely functioning as a biological nutrient (McDonough & Braungart, 2002). A simple design goal might be to create an entirely compostable product. The second approach emulates a product-as-a-service model. Consumers would buy (or "subscribe to") this product with the expectation of regularly returning it to the manufacturer and receiving a new one. The manufacturer can then take full responsibility for safely and effectively regenerating, recycling, or (at worst) disposing of the used product. An example of this model from the personal care product sector is the Preserve toothbrush, which is recycled and remanufactured after consumers return used toothbrushes by mail.

10.5 Research Priorities

The scientific research that has inspired us is quite far from the proposed realm of application. There is, at this time, no way to develop this strategy besides through the work of previously cited researchers and their colleagues. Nevertheless, a potentially important role for our partner companies is "upstream" engagement with researchers. Indeed, we believe the value of this proposal is not for immediate product development, but for initiating dialogue with a broader range of researchers, so as to help shape the technologies and applications that might later (or soon) be built upon their work.

11. Conclusions

Below we summarize our proposed strategies with respect to where they fall on our solution spectrum, whether it is at the chemical, formulation, or process level, or some combination thereof (Figure 15). We further provide a toxicity summary in addition to an implementation timeline, intended to aid our partners in determining which solutions are appropriate to pursue at this time and/or in the future.

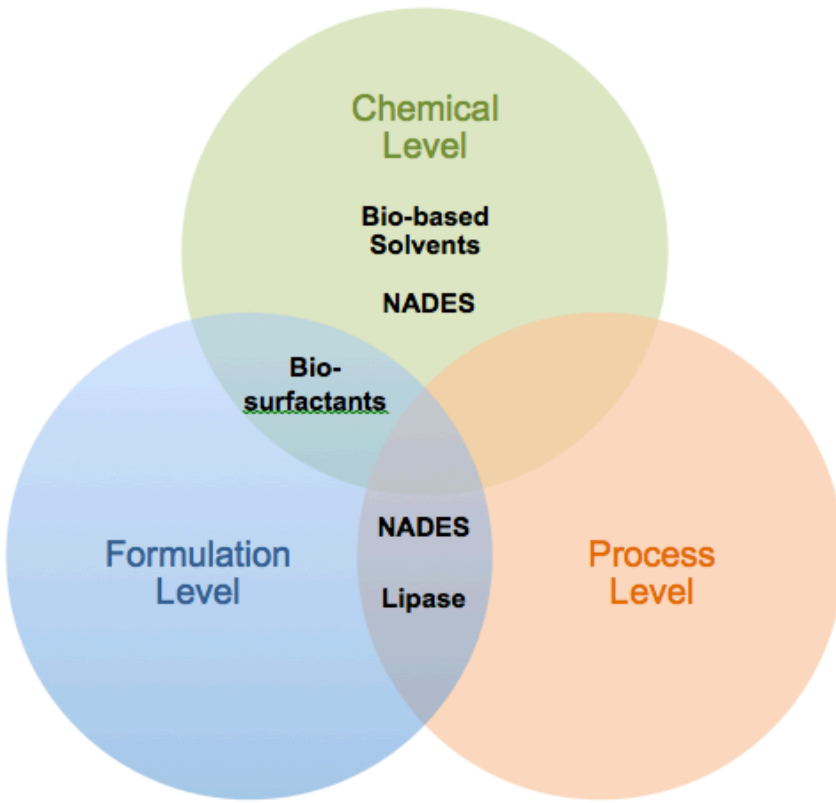


Figure 15. Proposed solutions and levels of application for oily soil removal at low temperature.

11.1 Chemical Level

Bio-based solvents and biosurfactants represent ingredients that may be directly added to current laundry detergent to either replace or enhance wash performance at low temperatures. They additionally have the added benefit of production from renewable feedstocks. NADES also have promise as laundry detergent ingredients by simply being added as additional solvents and/or imitating surfactant properties in the detergent. Drop-in chemicals or direct ingredient substitution using each of these strategies may be potentially useful to improve wash performance for oily stains at low temperatures while reducing the human health and environmental impact of the product.

11.2 Formulation Level

NADES and bio-based solvents are also promising as formulation level approaches. NADES acting as co-solvents or surfactants can potentially enhance wash performance at low temperatures by manipulating other detergent ingredients in the product formulation. The same is true for bio-based solvents that might have greater effectiveness through interaction with additional formulation ingredients. Some biosurfactants, like Surfactin, may also have additive effects with other laundry components, but further research is needed to determine if this is true. Enzymes are another strategy that can be implemented at the formulation level as they can interact with other laundry ingredients in various ways. Surfactants have been shown to decrease the time it takes for enzymes to operate which could potentially reduce the amount of time necessary for enzymes to work on oily stains and thus enhance their utility in laundry wash at low temperatures. Enzymes additionally interact synergistically with each other, making their combinations worth exploring in laundry detergent. Ultimately, each of these strategies provides interesting opportunities for exploration at the product formulation level.

11.3 Process Level

Interaction between different ingredients is also possible at the process level. NADES and enzymes could be used individually in a pre-treatment solution applied to laundry before washing, and the interaction between the two is also worth exploring. Enzymes show some promise with pre-treatment activity in conjunction with surfactants, and it may additionally be worth investigating whether biosurfactants and enzymes could work well together in this fashion. Our proposed cleaning object inspired by oil-adhesive surfaces could be used to keep oils away from the highly aqueous environment of laundry, and act to trap and remove these oils from the washing machine all together. This design concept would rely upon superoleophobic oil-adhesive surface technology, coated on the interior of a liquid permeable object. While purely hypothetical at this point, this concept may be used to spur further study and investigation into this type of technology. With more research, the oil-adhesive surface object along with our recommendations for pre-treatment may be promising novel process level approaches to removing oily stains at low temperatures in the future.

11.4 Hazard Summary

Method's 4x laundry detergent is a leading green cleaner in terms of hazards posed by existing ingredients. Most ingredients pose no or little to moderate risk to humans and the environment. Thus, thoughtful consideration of hazards should be made with respect to replacing and/or adding to the existing ingredient list, so that increased risk is not introduced at the expense of improved wash performance at low temperature. Although many toxicity data gaps exist for each of our strategies, we draw some conclusions based on the information we do have:

Enzymes likely pose little additional risk to human and ecological health. While enzymes were at one time an irritation/sensitization health risk in the occupational setting, reformulation and low consumer exposure risk have greatly reduced the overall threat. However, care should be taken to protect workers in the occupational setting to mitigate exposure intensity and duration. Enzymes additionally break down readily in the environment and have no existing indication of being ecotoxic.

Biosurfactants exhibit mild aquatic toxicity in addition to being potential skin and eye irritants. However, limited data reveals they score somewhat better than the currently used laundry detergent surfactants on the eco and human toxicity spectrum. They also biodegrade readily.

Bio-based solvents are also expected to degrade rapidly in the environment, but more research is needed on persistence and bioaccumulation. More data is additionally needed on human health risk, but there is currently no immediate concern.

NADES likely have varying eco and human toxicity concerns depending on chemical make-up, but toxicity gaps for individual components will need to be addressed further as part of this strategy. Additionally, screening for NADES toxicity at the mixture level will be an important part of this process, as NADES properties can change once components are combined. Biodegradability also varies depending on components, a factor that should be considered going forward.

Lastly, there are human toxicity and environmental impact concerns that need to be addressed for the proposed oil-adhesive surface strategy. The polymer coating is known to be toxic, the device itself would eventually need to be cleaned of oily soils, and the product poses a waste challenge at end-of-life. Additionally, it raises occupational health concerns on the manufacturing side of the lifecycle. While we propose some solutions to the sustainability problems, human toxicity concerns still need to be addressed.

11.5 Implementation Timeline

More robust evaluation is necessary for each of our recommended strategies, but they vary in terms of implementation timeframe (Figure 16). We think bio-based solvents could be implemented now due to BioAmber's ability to derive dialkyl succinates from succinic acid. Strategies that could be implemented in the near future include biosurfactants and enzymes. While it would took take some legwork to determine whether biosurfactant production is realistic using the Amyris yeast model, or whether it is viable to procure biosurfactants through some other avenue, these factors can be explored by our partners relatively soon, at least at the chemical substitution level. Likewise, steps in the near future can be taken to explore enzymes at the formulation and process levels. Both NADES and oil-adhesive surfaces, on the other hand, require more research before they could feasibly be implemented. A substantial amount of work is necessary to pursue these two strategies, but NADES show promise at all three solution levels.

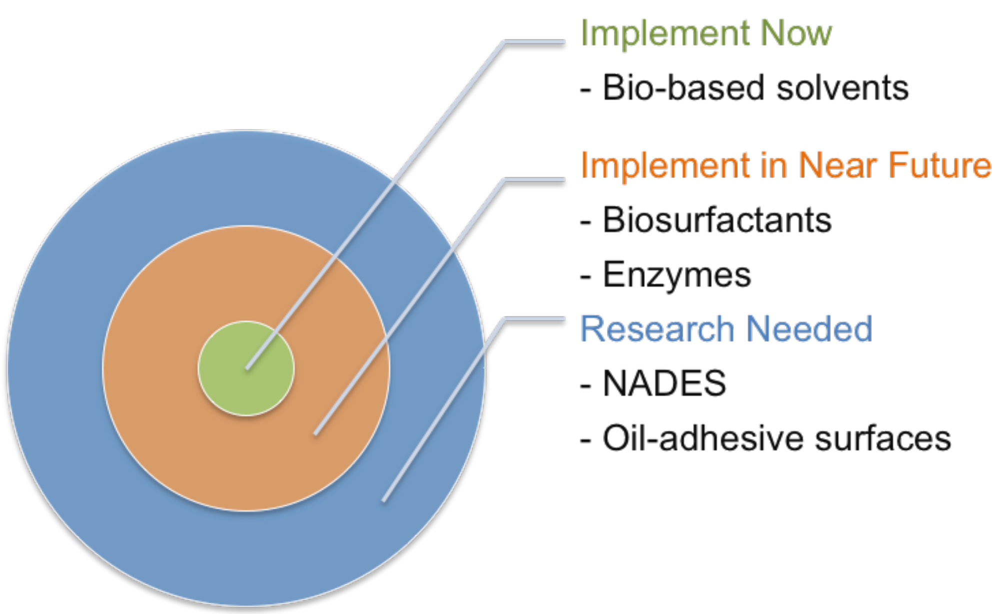


Figure 16: Opportunity map of timeframe for strategy implementation.

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13. About the Authors

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Kira Lou is in her second and final year of completing a Master's in Public Health degree, with a concentration in Environmental Health Studies at UC Berkeley. She also holds a Bachelor of Science degree in chemistry from Emory University. Most recently she has experience working for a scientific consulting firm, where she developed skills in reviewing the state of knowledge and evaluating the strength of evidence regarding different chemicals and exposures.

Julia Varshavsky is pursuing a PhD in Environmental Health Sciences at UC Berkeley's School of Public Health, where she also received an MPH in 2012. She specializes in exposure and risk assessment of chemicals found in consumer products. She previously received a BS in molecular environmental biology in 2004 (UC Berkeley) and worked in an ecotoxicology laboratory to help develop a microarray to improve metal detection in water. She also worked for five years at the Collaborative on Health and the Environment, an organization devoted to understanding and translating environmental health science.

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15. Supporting information

Appendix 1: Chemical information table

Table of identifiers, structures, and selected properties of chemicals investigated in this report: available online at <https://docs.google.com/spreadsheets/d/1rUDov70gywC8TGml0Gm6lIwJtUBK5hT9A2Ing0zUBSc/edit?usp=sharing>

Appendix 2: Hazard information table

Table of properties, classifications, and citations used in hazard assessment: available online at <https://docs.google.com/spreadsheets/d/1niGV8CI3bDT8bbyNxas8PP2bWKNvtTE-gTHvKtnU_Go/edit?usp=sharing>

Appendix 3: HSP Calculations

Calculation of Hansen solubility parameters for dialkyl succinate solvents: available online at <https://docs.google.com/a/berkeley.edu/spreadsheets/d/1k_9FiSxvXBwRV2-a-AJIz5pFA-aqeYu4cCcr3xCfWKY/edit?usp=sharing>