

Journal of Applied Microbiology

Yeast protein–surfactant complexes uncouple microbial electron transfer and increase transmembrane leak of protons

C.W. Podella, N. Hooshnam, S.M. Krassner,
M.G. Goldfeld

Published Online: Dec 1 2008

ORIGINAL ARTICLE

Yeast protein–surfactant complexes uncouple microbial electron transfer and increase transmembrane leak of protons

C.W. Podella¹, N. Hooshnam¹, S.M. Krassner² and M.G. Goldfeld¹¹ Advanced Biocatalytics Corporation, Irvine, CA, USA² Department of Developmental and Cell Biology, University of California, Irvine, CA, USA**Keywords**

degreasing, heat-shock proteins, microbial metabolism, proton transfer, soil decontamination, uncoupling, water, yeast.

Correspondence

Michael Goldfeld, Advanced Biocatalytics Corporation, 18010 Skypark Circle, #130, Irvine, CA 92614, USA.

E-mail: mgoldfeld@abiocat.com

2008/1003: received 13 June 2008, revised 28 August 2008 and accepted 3 September 2008

doi:10.1111/j.1365-2672.2008.03986.x

Abstract

Aims: To explore the combined effect of yeast proteins and surfactants on bacterial metabolism.

Methods and Results: Protein-rich cell-free supernatant from heat-shocked yeast *Saccharomyces cerevisiae* was combined with certain synthetic surfactants. These blends affected the metabolism of a Polyseed inoculum of aerobic bacteria, accelerating CO₂ production and consumption of nutrients from a sterile nutrient broth solution, without a concomitant accumulation of biomass. It is suggested that in the presence of the yeast protein–surfactant complexes, bacterial electron transport is uncoupled from biomass accumulation. The ‘uncoupling hypothesis’ is supported by experiments with model membranes, in which the same complexes induced proton leak similar to standard chemical uncouplers, such as dinitrophenol, indicating that uncoupling may occur at the stage of generation of the transmembrane pH gradient as the driving force for ATP production.

Conclusions: Yeast protein–surfactant complexes behave as uncouplers of oxidative metabolism in bacteria and appear to do so by increasing proton permeability of membranes.

Significance and Impact of the Study: Yeast proteins may be of interest as nontoxic, environmentally benign and economically sound agents accelerating oxidative bacterial metabolism while uncoupling it from biomass accumulation. There are actual and potential implications in waste water/soil decontamination, degreasing and other environmental technologies.

Introduction

Yeast responds to stress conditions by producing several low-molecular weight proteins that are partially released into the fermentation medium or become available after the yeast cell lysis. These amphitropic proteins were shown to associate with membranes, and recent studies indicate that they may play an important role in membrane quality control and the maintenance of membrane integrity under stress conditions (for an up-to-date review see Nakamoto and Vigh 2007). Although referred to as ‘heat-shock proteins’, it appears that yeast produce a similar blend of proteins under other stress conditions

besides excessive heating, such as starvation and oxidative stress. In recent years, yeast stress-induced proteins have been used for water decontamination as commercially available blends with certain surfactants and some other adjuvants (Podella and Hauptmann 2004). These products drastically reduce interfacial tension in water/hydrocarbon and water/oil systems. They were successfully applied for degreasing sewer lines, cleaning of soils contaminated with petroleum oil, reducing biofilming in reverse osmosis membranes, deodorization treatment and in several other related arenas (Baldrige and Podella 2005, 2006a,b; Podella and Hauptmann 2004). These complexes appear to affect the growth rate of

normal environmental microflora, resulting in a synergistically enhanced bacterial decontamination of water and soil (Podella and Goldfeld 2006). Enhancement of the cleaning power of the complex in the presence of natural microflora, e.g. in sludge, raises the question about the mechanism of such a synergism. This question is addressed in the present study by two essentially complementary ways: exploration of the effect of the yeast protein–surfactant blends on the rate of bacterial metabolism under strictly controlled conditions, and measurements of proton transfer in model membranes in the presence of the same materials.

In the first part of the study, we observed that in the presence of the heat-shocked yeast protein–surfactant complexes, the production of carbon dioxide and the consumption of nutrients by bacteria were accelerated with a lower than expected concomitant acceleration biomass growth, when compared with control without additives. This result suggests that there is at least a partial uncoupling of electron transfer from energy production in the form of transmembrane proton gradient that eventually leads to ATP-dependent biomass growth. As a result, the electron transfer is not fully associated ('coupled') with biosynthetic reactions that require ATP, so that a reduced biomass growth takes place, while nutrients are consumed at an increased rate.

In an attempt to obtain independent support for the above 'uncoupling hypothesis', and to further specify the metabolic level at which such an uncoupling may occur, we then explored the effect of the same yeast-derived materials on the kinetics of proton leak across a model hydrophobic membrane. We compared these observed effects with those exerted by standard protonophoric uncouplers.

It was earlier shown (Kocherginsky *et al.* 1989, 1991) that nitrocellulose filters impregnated with hydrophobic materials, such as fatty acid esters or natural oils, mimic some essential properties of biological membranes, such as electrical impedance, penetrability for ions, water and neutral molecules (when scaled to their thickness, as compared with the bilayer nature of cell and/or organelle membranes). Here, we measured the kinetics of pH-gradient driven passive proton leak across such a membrane in the presence of some standard uncouplers of oxidative phosphorylation, as well as in the presence of the yeast proteins and their complexes with synthetic surfactants.

As standard uncouplers, we tested 2,4-dinitrophenol (DNP) and lauric acid. DNP is known to uncouple oxidative phosphorylation by carrying protons across mitochondrial and bacterial membranes, thereby suppressing the formation of ATP, short-circuiting and thus accelerating electron transfer. Cells partially compensate for the decreased yield of ATP by oxidizing more nutrients, con-

suming more oxygen and producing more carbon dioxide. It was previously shown that uncoupling by DNP results in accelerated water decontamination, among other effects (Shah *et al.* 1975; Low and Chase 1998). More recently, Chen *et al.* (2004) and Ye and Li (2005), following the same line of reasoning, applied another chemical uncoupler, tetrachlorosalicylanilide, to reduce activated sludge production in bench experiments.

Lauric acid, as a weak acid with a hydrophobic tail that renders its membrane-soluble, also acts as a protonophore-type uncoupler (Garlid *et al.* 1996).

In the experiments with artificial membranes, we demonstrate that the heat-shock protein complexes indeed accelerate proton transfer across membrane with an effect comparable to the standard uncouplers: DNP and lauric acid.

Materials and methods

Heat-shocked yeast proteins

The yeast *Saccharomyces cerevisiae* (from Red Star Yeast Co., Milwaukee, WI) was cultivated under continuous aeration and agitation between 30°C and 35°C and at a pH range of 5.2–5.6, until a minimum level of 4% dry mass was attained. At the conclusion of the fermentation process, the fermentation product was heated to 50°C for 8 h to induce a heat-shock response followed by autolysis of the yeast cells, thus releasing the proteins into the growth media. After autolysis, the yeast fermentation mass was centrifuged to remove unlysed cells and debris, and the supernatant ('ferment') was blended with surface-active agents, while the pH was adjusted to 4.0–4.6. The protein content in ferment was typically 35 mg ml⁻¹.

In some experiments, the autolysis product was additionally homogenized using a Manton-Gaulin High Pressure Homogenizer at 7000 psi for three cycles, after which the yeast fermentation product was centrifuged to remove cell debris and the supernatant (labelled as 'disrupted-cell ferment') was blended with surface-active agents in the same way as above. In some experiments, the low-molecular weight proteins were separated from the mixture using an Amicon Ultra centrifugal filter device (Millipore) with 10 kD cut-off. The composition of the blend is shown in Table 1. The following reagents were added to the protein solution: propylene glycol from Dow Chemical, ethoxylated C9-11 alcohol 3 mole EO from Tomah Reserve (Milton, WI); sodium lauryl ethoxy sulfate (SLE), from Pilot Chemical (Cincinnati, OH), sodium dioctylsulfosuccinate (DOSS) from Cytec Industries, and hexylene glycol from the Shell Oil Company.

The blends as described in Table 1 were then stored as stock solutions for further dilution, and the final

Table 1 Typical composition of various versions of the products used in this study, in per cent by volume

	Ferment	Propylene glycol	Ethox. alcohol 6EO	SLE (60%)	DOSS (75%)	Hexylene glycol
Concentrated ferment	77.67%	21.09%	0%	0%	0%	0%
Accell [®] 3	54.37%	14.76%	22.44%	7.48%	0%	0%
Accell [®] STR	48.54%	13.18%	0%	0%	25.00%	12.50%

concentration was listed in parts per million (ppm) in solution added to the membrane cell, or to the bacterial growth medium.

Experiments with bacteria

Two sets of tests were conducted in this part of the study.

In the first one, a sterile nutrient broth solution (Bacto Tryptic Soy Broth, from Becton Dickinson & Co.) was inoculated with Polyseed, a standard blend of aerobic bacteria from InterLab, approved by the EPA for conducting biological oxygen demand (BOD) tests. Any suspended solids or particulate matter that developed during the course of study is considered as biomass produced as a result of the assimilation of nutrient carbon source.

A 2-l reactor, Applikon Bio Console, Model ADI 1025 and an Applikon BioController, Model ADI 1010 (Applikon (Netherlands) Bio) were used in these experiments. Incoming air was first sparged through a 1.59 N NaOH solution and then through twice deionized water to remove all carbon dioxide from the aeration source. The bioreactor exhaust air was then sparged through a 1.5 N NaOH trap and the amount of carbon dioxide respired in the bioreactor during the test period was then determined as described below.

Tryptic soy broth (TSB) solution was prepared by adding 72 g of sterile 10% TSB concentrate to 2.40 l of deionized water in a 4-l beaker. Two capsules of Polyseed inocula were added to the nutrient solution. The inoculated nutrient was warmed up to, and maintained at 30°C, with continuous agitation for 14 h. Prior to transferring the nutrient broth to the bioreactor, the solution was filtered through four layers of sterile cheesecloth to remove the filler used as a substrate for the dried bacteria in the Polyseed. Two litres of the nutrient solution were added into the bioreactor. The 'treated' samples contained 10 mg l⁻¹ of the test composition (unless different levels as noted), added to the nutrient. In 'control', the same volume of deionized water was added to the nutrient solution instead of the test composition.

The bioreactor was then sealed and carbon dioxide-free air was sparged, whereas the bioreactor temperature was maintained at 30°C with agitation. CO₂ in the exhaust air was captured by 1.5 N NaOH. The nutrient was sampled

at 0 h and again at the conclusion of the study (typically, after 4 h of growth). Filtered and unfiltered nutrient samples were analysed for total organic carbon (TOC) using a Shimadzu Total Organic Carbon Analyzer, Model TOC-5000A.

Captured CO₂ was calculated by potentiometric titration of NaOH with HCl solution at the end of the test. TOC and organic carbon in solution before and after the test were determined by converting the organic matter to CO₂ and conducting the titration in the same way as indicated above. The level of biomass carbon was determined by the difference between the TOC and soluble OC.

Carbon mass balance was estimated using the equation:

$$\text{Nutrient C Consumed} = \text{Biomass C Increase} + \text{C Respired as CO}_2$$

Table 2 shows that such a balance was indeed maintained within a reasonable margin.

In order to characterize the extent of energy coupling, we used two uncoupling parameters (UP), both presenting the ratio of carbon processed in either nutrient consumption (total electron transfer), or carbon dioxide formation (uncoupled electron transfer), to carbon used in biomass increase (coupled electron transfer).

In the second set of experiments with bacteria, bacterial growth was estimated by measuring the total suspended solids (TSS) in a bulk culture through turbidity measurements with a Hach DR/2400 spectrophotometer, at intervals of 0, 24 and 48 h. In the same samples, total plate counts were also performed (colony forming units, CFU). For these measurements, 0.1 ml samples from the bulk culture were spread onto total plate count agar in a Petri dish and incubated at 30°C for 48 h, with the number of CFU presented per 1 ml of the sample (CFU ml⁻¹).

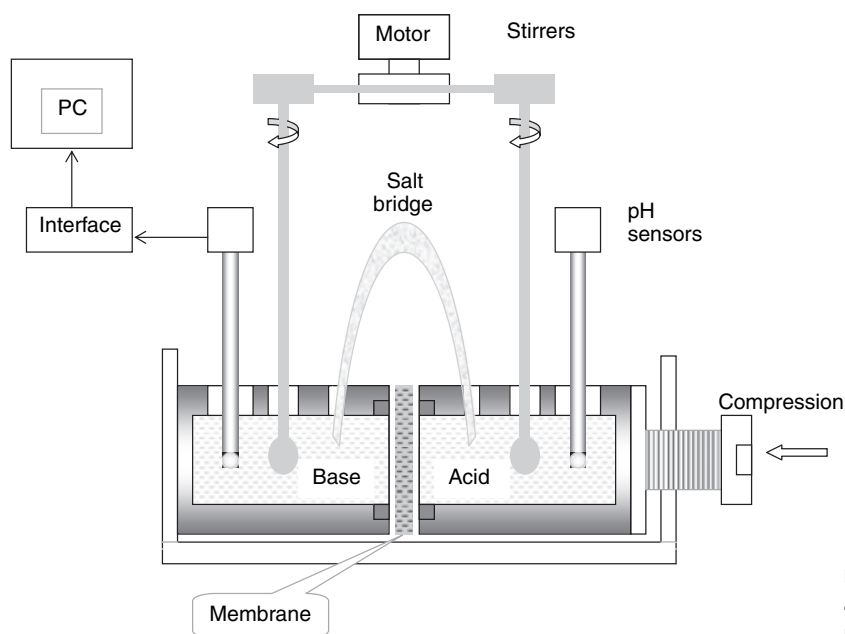
Experiments with artificial membranes

Measurements were conducted using a two-chamber set essentially as previously described (Kocherginsky *et al.* 1989, 1991), with minor variations. Its schematic is shown in Fig. 1. 20 ml of nonbuffered 0.1 mmol l⁻¹ solutions of HCl and NaOH, with pH values about 2.5 and

Table 2 Mass balance of aerobic fermentation in control and several experiments

	Control	1	2	3	4	5
Nutrient TOC (mg l ⁻¹)	175.3	353.0	430.0	455.9	356.7	347.0
Biomass TOC (mg l ⁻¹)	142.8	147.2	238.3	250.2	200.6	184.2
CO ₂ (mg l ⁻¹)	49.5	198.0	220.5	252.0	211.5	157.5

1, nonautolysed ferment; 2, autolysed ferment; 3, disrupted-cell ferment; 4, disrupted-cell ferment protein fraction <10 kD; 5, heated disrupted-cell ferment protein fraction <10 kD.

**Figure 1** Schematic of the experimental two-chamber set for proton transfer across the membrane.

12.2, respectively, were placed in each chamber. The pH values were continuously monitored with pH sensors (computer-interfaced Vernier LABPRO).

The membrane was prepared by immersing a Millipore nitrocellulose ultra-filter (47 mm diameter, 0.1 mm thick, pore size of 0.22 μm) in Star Pure Original olive oil for 5 min, then pressed between folds of filter paper to remove the excess oil. The prepared membranes were mounted between the two chambers by compression.

The pH sensors, as well as mechanical stirrers, were placed in each of the two chambers. The chambers were connected with a saturated KCl salt bridge to compensate for electrical potential generated in the course of proton transfer. The pH recording lasted for up to 25 h after addition of the uncoupler; further rates of pH shift were too slow for convenient observations.

The uncouplers [DNP and lauric acid (both from Sigma-Aldrich) or the yeast protein complex] were introduced as a 10-ml aliquot of known concentration into the acidic arm of the set. Our experiments showed, however, that the results would be essentially the same, regardless of which side of the two-chamber cell was

loaded with uncoupler. As a control, 10 ml of distilled water was added to both solutions at the beginning of recording. Several concentrations of the complex, or its protein-only component, or contributing surfactants, were tested in the above setting. All the experiments were conducted at least in triplicate.

Results

Effect of the yeast protein–surfactant complex on microbial metabolism

Figures 2 and 3 show the results of measurements of biomass accumulation and carbon dioxide release by bacterial cultures, presented as per cent of untreated control. In Fig. 2, the uncoupling parameter 1 (UP1) represents the ratio of the uncoupled electron flow to the coupled one. UP1 increases in the presence of the yeast protein–surfactant complex by 3–3.5 times. In Fig. 3, the uncoupling parameter 2 reflects the ratio of the total electron flow to the uncoupled one. UP2 increases 1.5–1.8 times.

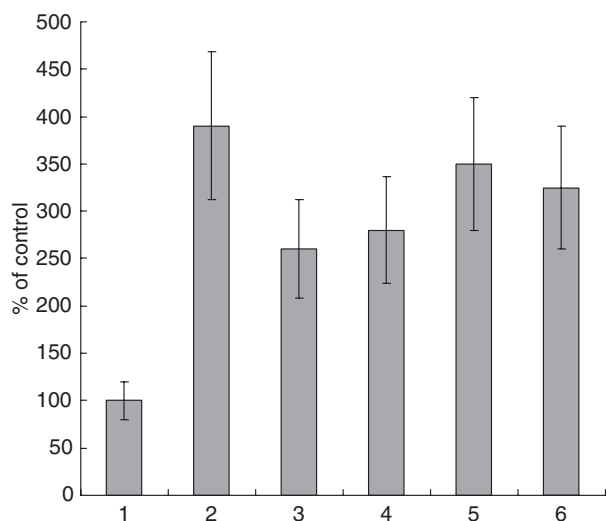


Figure 2 Effect of various additives on the UP1: the ratio of carbon processed in CO₂ formation to carbon used in biomass increase, ΔCO_2 carbon/ Δ Biomass carbon in per cent of untreated control (1). (2) Nonautolysed ferment; (3) Autolysed ferment; (4) Disrupted-cell ferment; (5) Disrupted-cell ferment <10 kD; (6) Heated disrupted-cell ferment <10 kD.

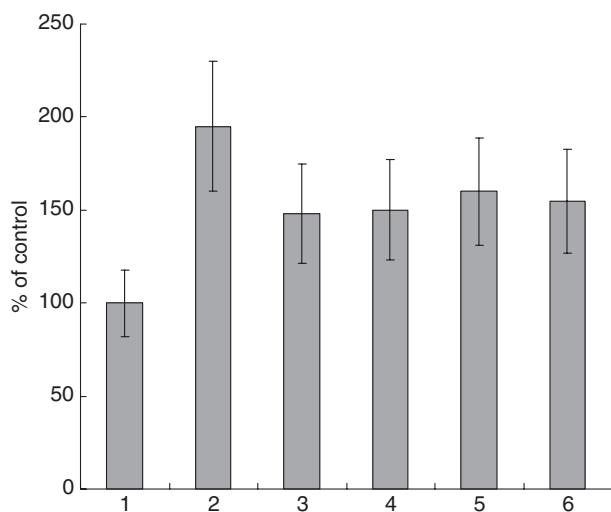


Figure 3 Effect of various additives on the UP2: the ratio of carbon processed in CO₂ formation to carbon used in biomass increases in per cent to the untreated control. 1–6 are the same as in Fig. 2.

In the second set of the experiments with bacteria, it was likewise found that, at least within the first 24 h of growth, both the rates of the TSS growth, and CFU count growth were reduced in the presence of the complex. This is shown in Fig. 4 as per cent of untreated control. Eventually, both reached the similar level, presumably, because of the exhaustion of nutrients.

Effect of yeast protein–surfactant complex on the membrane transfer of protons

Typical results from multiple replicates are shown in Fig. 5. In controls, without protonophoric additives, the membrane provides a stable barrier, preventing the two chambers from exchanging protons: the pH does not shift for a long time in either compartment. When standard protonophoric uncouplers, DNP or lauric acid, were added, the pH in the alkaline half-cell decreased because of proton transfer from the acidic arm, whereas the pH value in the acidic compartment remained essentially unaffected when presented at the same scale.

Essentially, uncouplers drastically accelerated the proton leak from the acidic compartment, thus decreasing the pH in the alkaline solution (down to about pH 7), whereas no substantial pH changes in the acidic compartment were observed.

The yeast protein–surfactant complexes act in a manner similar to both standard protonophoric uncouplers, DNP and lauric acid.

The rate of the pH drop was dependent on the complex concentration as shown in Fig. 6. Here and further on the ordinate is the pH drop slope taken during the second hour of the observed kinetics, to avoid the steep initial pH drop which is hard to quantify. The graph may serve as a guide to the most appropriate dilution for applying the complex, from both functional and economic standpoints.

The surfactant component of the complex displayed a much weaker effect, than the entire blend, while the proton gradient suppression by the protein ingredient ('ferment') without synthetic detergents was about as strong as that of the entire blend (Fig. 7).

The relative protonophoric efficiency of the complex, as well as the effect of the contributing surfactants and standard uncouplers is presented in Fig. 8.

For application purposes, it was important to see how resistant the complex is to extreme conditions, such as heating. According to the proton transport criteria, we found it to be quite stable: boiling the complex solution (at the same dilution used in proton gradient experiments), for 3 h, did not diminish its protonophoric effect ('preheated complex' in Fig. 8). This is in line with the measurements of surface activity that was unaffected under the same heating conditions (measured as the pendent drop in oil/water interface tension, unpublished data).

Discussion

The data on the effect of yeast protein–surfactant complexes on bacterial metabolism (Figs 2 and 3) clearly indicate a pronounced uncoupling of bio-oxidative reactions from biomass accumulation. In the presence of

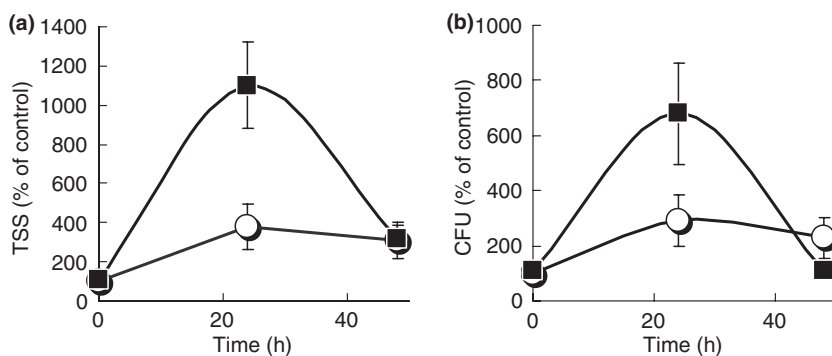


Figure 4 Biomass accumulation according to turbidimetric measurements of the TSS, in per cent of control (a, 100% TSS is 115 mgC min⁻¹), and change in CFU count (b, control 100% is 1.9 × 10⁶ CFU ml⁻¹). ■, control; ○, treatment with the yeast protein-surfactant complex.

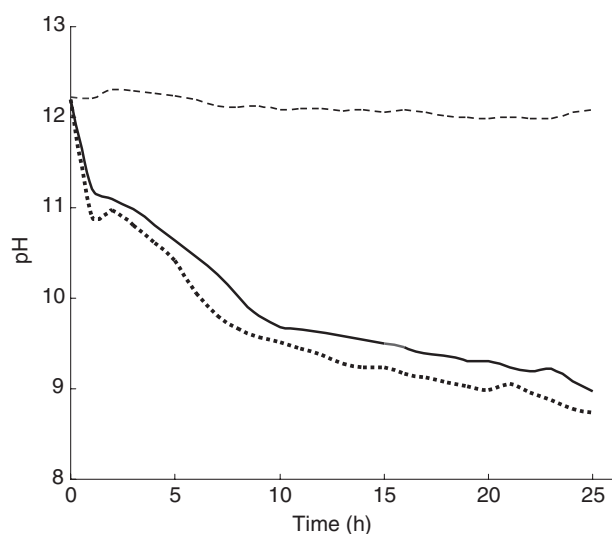


Figure 5 Kinetics of pH drop in the alkaline half-cell: control (---); DNP, 0.1 mmol l⁻¹ (.....) and yeast protein-surfactant complex, 30 ppm (—).

these complexes, the nutrient oxidation to carbon dioxide is diverted to a path, which does not result in concomitant growth of biomass. As expected, the UP1 (Fig. 2) which represents the ratio of the *uncoupled* electron flow to the *coupled* one, is more sensitive to the presence of the yeast protein-surfactant complex, than UP2 (Fig. 3) – the ratio of the *total* electron flow to the *coupled* one. Independent data on accumulation of TSS and bacteria counts (Fig. 4) corroborate these findings, also demonstrating the decrease in biomass accumulation in the presence of yeast protein-surfactant complex in the first 24 h of incubation. Some bacteriostatic effect of yeast proteins were earlier documented by Comitini *et al.* (2005), but the character of the metabolic response in the latter study and in our experiments was very different.

The experiments with artificial membranes shed some light on the possible mechanism of such an uncoupling. As it is well known, many standard uncouplers of oxidative phosphorylation, such as DNP, are rather hydropho-

bic weak acids capable of shuttling protons across biological membranes along the pH gradient. This way, they dissipate the transmembrane proton gradient which is, according to Mitchell chemo-osmotic theory, the driving force of phosphorylation. Lack of ATP prevents biosynthetic processes and hence accumulation of biomass, while electron flow associated with aerobic nutrient oxidation to carbon dioxide is liberated from phosphorylation control and thus accelerated.

Data obtained with artificial membranes show that the yeast protein-surfactant complexes (as well as yeast proteins by themselves) increase the proton leak across the hydrophobic liquid, porous polymer-supported membrane essentially in the same manner as such standard uncoupling agents as dinitrophenol or lauric acid. That is displayed in Figs 5–8: the pH gradient preset on the membrane separating an acidic (pH *c.* 2) and alkaline (pH *c.* 10) solutions, in the absence of proton-shuttling additives is stable practically indefinitely. However, in the presence of both DNP or lauric acid, as well as the yeast proteins and their complexes with synthetic surfactants, the pH of the alkaline solutions decreases, and the kinetics of this pH drop can be taken as the measure of uncoupling activity. The pH drift in the alkaline solution was initially fast, but then slowed down and usually stopped almost completely after reaching pH 7–8. At the same time, the pH value of the acidic solution remained practically unchanged at the same sensitivity scale. This behaviour is not unexpected in the setting used. Indeed, a simple simulation of the anticipated shifts in pH values in both compartments, based on the passive diffusion model for proton transfer driven by the transmembrane gradient of proton concentrations, results in the following equations for the pH value change in both compartments:

$$\begin{aligned} \text{pH}_{\text{alkaline}} &= -\log([H^+]_{\text{o,alk}} + DA[H^+]_{\text{acid}}t); \text{pH}_{\text{acidic}} \\ &= -\log([H^+]_{\text{o,acid}} + DA[H^+]_{\text{acid}}t) \end{aligned}$$

where *DA* is a diffusion parameter (depends on the diffusion coefficient, membrane thickness and cross-section area) and *t* is the time of transfer. Crude simulation gives

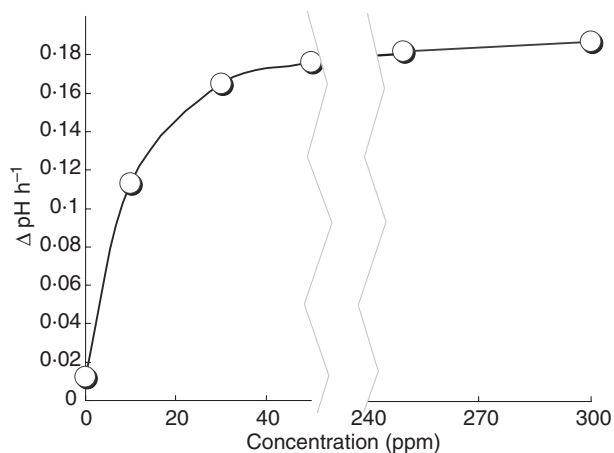


Figure 6 The slope of pH drop in the alkaline compartment as a function of the concentration of the yeast protein-surfactant complex.

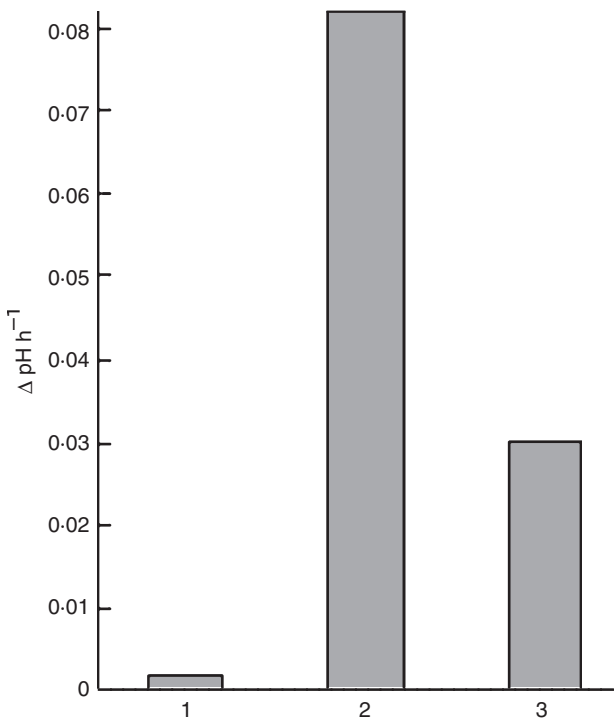


Figure 7 The slope of pH change in the alkaline compartment is depicted as the average pH drop per hour over first 8 h of pH recording in the alkaline compartment: The pH change with 'ferment' (2) and surfactant (3) (both at 30 ppm) are compared with that in control (1).

the graphs shown in Fig. 9 for a few values of DA arbitrarily chosen to fit the observed kinetics to the order of magnitude in the time scale. The pH values in the acidic half-cell do not appreciably change, and the pH drift in

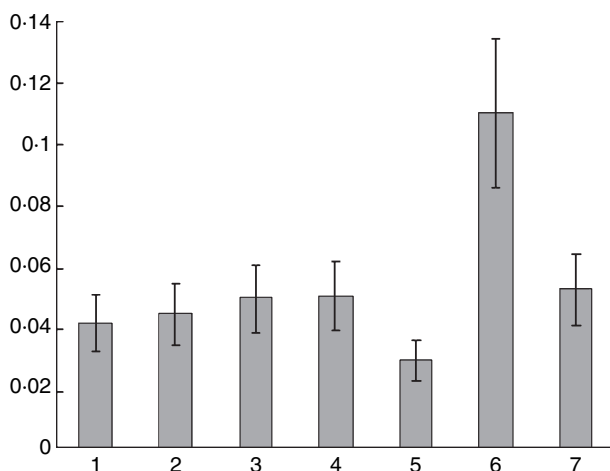


Figure 8 Comparison of the effect of various additives on the slope of pH drop in the alkaline compartment of the proton transfer membrane set. (1) lauric acid, 0.1 mmol l^{-1} ; (2) DNP, 0.1 mmol l^{-1} ; (3) yeast protein-surfactant complex; (4) same as (3), but preheated; (5) surfactant without the yeast protein; (6) STR; (7) concentrated ferment (all 3-7 at 30 ppm).

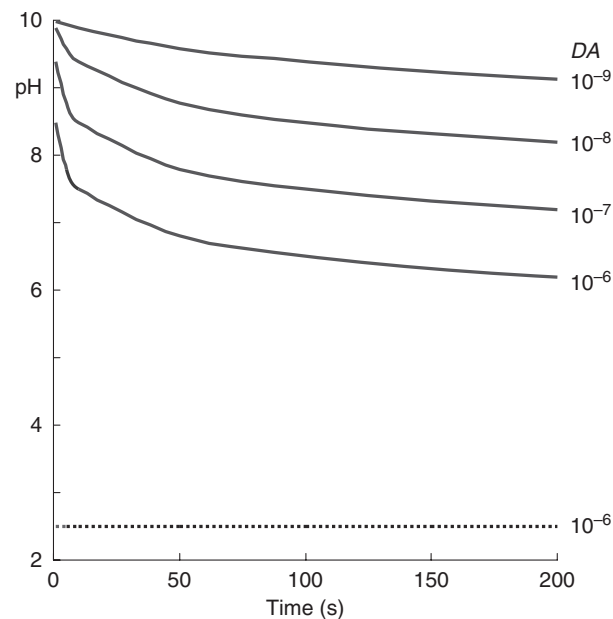


Figure 9 Simulation of the expected kinetics of pH shifts at different values of the diffusion parameter DA . Bottom line – pH in the acidic half-cell, all other curves – in the alkaline half-cell.

the alkaline compartment slows down after a while, long before equal pH values settle down in both compartments. Those are the essential features which we observed experimentally.

It is important that in these experiments with artificial membranes, the yeast proteins, even in the absence of

synthetic surfactants, induced transmembrane proton leak at least as efficiently as protein–surfactant complexes, while surfactants alone provided only marginal pH drift (Fig. 7). Therefore, the protein component of the complex is critical for the protonophoric uncoupling effect.

Summarizing the results obtained with both approaches: experiments on the rate of microbial metabolism under strictly controlled conditions, and kinetic measurements of the passive proton transfer driven by the pH gradient across an artificial membrane, we see that in both situations, the yeast protein–surfactant complexes display a behaviour typical for standard protonophoric uncouplers. It is clearly seen from these data, that there is a well-pronounced uncoupling between nutrient oxidation (electron flow) and biomass accumulation.

Regarding implications of the phenomena described in this study, our entire focus was on the actual and potential uses of the yeast proteins in environmental cleaning and remediation technologies. However, yet another potentially exciting aspect of the metabolic uncoupling effect of yeast protein–surfactant complexes may lie in the therapeutic significance of agents with this type of biological activity, as was recently described in mice with experimental and genetic disorder of cardio-vascular functions (Bernal-Mizrachi *et al.* 2002). Very early attempts to apply some chemical uncouplers in medical field failed, because these uncouplers were highly toxic and ‘eliminated patients together with the disease’. Yeast proteins could provide a totally benign, nontoxic and safe alternative to that approach.

Conclusions

The major conclusion for this study is that the nature of the synergistic enhancement of the decontaminating effect of the yeast protein–surfactant complexes in natural environments may be, at least partially, because of its uncoupling of electron-transfer metabolism in a natural blend of bacteria, from the energy accumulating steps of generating the transmembrane pH gradient, and eventually ATP synthesis. Such an uncoupling results in the acceleration of the electron transfer associated with increased consumption of nutrients and carbon dioxide production, hence increasing the rate of processing of hydrophobic organic contaminants, such as petroleum oil or grease, without a concomitant increase in bacterial biomass. At the same time, the complexes act as a powerful surfactant, drastically reducing the interface tension between the hydrophobic particles to be processed and eliminated from contaminated media. That facilitates the processing of contaminating microparticles of petroleum, fats and oils by bacteria. Moreover, the synergism most likely consists not only in the acceleration of bio-oxidation *per se*,

but also the conversion of contaminating hydrophobic compounds, by enzymatic hydrolysis and partial oxidations, into heteropolar surface-active species, thus additionally facilitating further processing of hydrophobic contaminants.

Acknowledgements

The authors would like to acknowledge the contribution of Adriana Garcia and Barbara Granger in some of the experiments described in this study.

References

- Baldrige, J.W. and Podella, C.W. (2005) Changing the nature of surfactants. *Ind Biotechnol* **1**, 288–291.
- Baldrige, J.W. and Podella, C.W. (2006a) Septic System Cleaning. US Patent 20060201877.
- Baldrige, J.W. and Podella, C.W. (2006b) Reduction of Surface Tension, Interfacial Tension, Critical Micelle Concentration Using a Protein-Based Surfactant Synergist. US Patent 20060270583.
- Bernal-Mizrachi, C., Weng, S., Li, B., Nolte, L.A., Feng, C., Coleman, T., Holloszy, J.O. and Semenkovich, C.F. (2002) Respiratory uncoupling lowers blood pressure through a leptin-dependent mechanism in genetically obese mice. *Arterioscler Thromb Vasc Biol* **22**, 961–968.
- Chen, Y.X., Ye, F.X. and Feng, X.S. (2004) The use of 3,3',4'5-tetrachlorosalicylanilide as a chemical uncoupler to reduce activated sludge yield. *J Chem Technol Biotechnol* **79**, 11–116.
- Comitini, F., Ferretti, R., Clementi, F., Mannazzu, I. and Ciani, M. (2005) Interactions between *Saccharomyces cerevisiae* and malolactic bacteria: preliminary characterization of a yeast proteinaceous compound(s) active against *Oenococcus oeni*. *J Appl Microbiol* **99**, 105–111.
- Garlid, K.D., Orosz, D.E., Modryansky, M., Vassanelli, S. and Jersek, P. (1996) On the mechanism of fatty acid-induced proton transport by mitochondrial uncoupling protein. *J Biol Chem* **271**, 2615–2620.
- Kocherginsky, N.M., Goldfeld, M.G. and Osak, I.S. (1989) Electron-proton coupled transport across biomimetic polymer-supported liquid membrane. *J Memb Sci* **45**, 85–98.
- Kocherginsky, N.M., Goldfeld, M.G. and Osak, I.S. (1991) Photostimulated coupled transport of electrons and protons across quinone-doped liquid polymer-supported biomimetic membrane. *J Memb Sci* **59**, 1–14.
- Low, E.W. and Chase, H.A. (1998) The use of chemical uncouplers for reducing biomass production during biodegradation. *Water Sci Technol* **37**, 399–402.
- Nakamoto, H. and Vigh, L. (2007) The small heat shock proteins and their clients. *Cell Mol Life Sci* **64**, 294–306.

- NSF International Certificate # 3U141-01, March 5, 2007.
- Podella, C.W. and Goldfeld, M.G. (2006) Sludge reduction and process improvements by an environmentally friendly, cell-free, biologically active composition. In *Proceeding of the 40th Western Regional ACS Meeting*, Anaheim, CA, pp. 215.
- Podella, C.W. and Hauptmann, N. (2004) Altering Metabolism in Biological Processes. US Patent 20040180411.
- Shah, P.M., Snyder, R.A., Constantinides, A., Wang, S.S. and Vieth, W.R. (1975) Enhancement of specific waste water treatment by the uncoupler 2,4-dinitrophenol. *J Food Sci* **40**, 302–305.
- Ye, F.X. and Li, Y. (2005) Uncoupled metabolism by chemical uncoupler and oxic-settling-anaerobic combined process to reduce excess sludge production. *Appl Biochem Biotechnol* **127**, 187–199.