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Detecting and differentiating *Escherichia coli* strain TOP10 using optical textures of liquid crystals

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We report a method for detecting *Escherichia coli* using a nematic liquid crystal (LC), 4-cyano-4′-pentylbiphenyl (5CB). Among three *E. coli* strains tested, TOP10 strain grown on agar plates induces a homeotropic orientation of LCs whereas DH5α and JM109 strains do not. This results in a clear distinction in the optical appearance of LCs as either uniformly dark or bright under polarised light. The LC-based method provides a simple, rapid and low-cost method of identifying *E. coli* strains.

Keywords: *Escherichia coli*; optical sensor; 5CB; differentiate

1. Introduction

Escherichia coli is a widely used indicator of water and food contamination. Some *E. coli* strains can cause a wide variety of intestinal and extra-intestinal diseases such as diarrhoea, urinary tract infection and meningitis. Therefore, developing an efficient method to detect and differentiate *E. coli* strains is essential for public health.

Synthetic liquid crystals (LCs) are receiving increasing attention for their potential biosensor applications. The advantages of LC biosensors include high sensitivity, low cost, ease of use and their label-free nature. Recent studies have shown that LCs can transduce and amplify the presence of proteins, phospholipids, DNA, cells and viruses into optical readouts visible to the naked eye [1–16].

However, to the best of our knowledge, the study of LC responses to bacteria such as *E. coli* is still very limited [17]. For example, Sivakumar *et al.* [17] used monodispersed, polymer-coated LC droplets to distinguish Gram-positive and Gram-negative bacteria. The ordering transition of LC droplets is observed in the presence of *E. coli* (Gram-negative bacteria) because the presence of lipid in the outer membranes of these bacteria disrupts the orientation of LC. However, this method cannot be used to differentiate *E. coli* of different strains.

Therefore, a new method was developed in this study to detect the presence of Gram-negative bacteria *E. coli* and to differentiate different strains of *E. coli* by using the orientational responses of a nematic liquid crystal, 4-cyano-4′-pentylbiphenyl (5CB). The mechanism by which LCs distinguish different *E. coli* strains is possibly due to the unique LC responses to different extracellular polymeric substances (EPS) secreted by the respective *E. coli*. This LC-based *E. coli* identification method has potential application for rapid and simple differentiation of *E. coli* strains.

2. Materials and methods

2.1 *Materials*

Glass slides were obtained from Marienfeld (Germany). *N, N*-dimethyl-*n*-octadecyl-3-aminopropyltrimethoxysilyl chloride (DMOAP) and poly(ethylene imine) (PEI) were purchased from Sigma Aldrich (Singapore). Nematic liquid crystal, 4-cyano-4′-pentylbiphenyl (5CB), was purchased from Merck (Singapore). Non-pathogenic *E. coli* was kindly provided by Professor Lianhui Zhang of the Institute of Molecular and Cell Biology (IMCB), Singapore, and Dr Jianzhong He of the National University of Singapore. All solvents used in this study were HPLC grades. Deionised water was obtained from a Milli-Q system (Millipore, USA). Phosphate-buffered saline (PBS) was diluted from stock buffer solution to a final concentration of 10 mM (pH 7.4). Other 10 mM buffers (pH = 4, 6, 8, 10) were prepared using acetic acid, 2-(*N*-morpholino)ethanesulfonic acid (MES), tris(hydroxymethyl)aminomethane (Tris) and sodium carbonate, respectively.

2.2 *Preparation of* E. coli *solutions*

Non-pathogenic *E. coli* was incubated in culture flasks using standard Luria–Bertani (LB) broth. (Ten grams of tryptone, 5 g of yeast extract and 10 g of sodium chloride were dissolved in 1 litre of deionised water. The pH of the solution was adjusted to 7.5 using sodium hydroxide and the broth was autoclaved at 121°C for 20 min.) After growth overnight, *E. coli*

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was harvested by centrifugation (Eppendorf 5810R, Germany, 10,000 rpm, 10 min.) and washed three times with PBS. The concentration of *E. coli* was determined by measuring the optical density at 600 nm (OD_{600}) using a UV/Vis spectrometer (Cary 50, Varian, Australia). The stock solution was diluted to a final concentration with fresh buffers. Alternatively, *E. coli* was incubated on agar plates overnight at 37°C and then moved to a 4°C refrigerator before use. The *E. coli* solution was prepared by picking an *E. coli* colony from the agar plate, dissolving it in buffer and diluting it to the desired concentration.

2.3 *Preparation of PEI-coated and DMOAP-coated glass slides*

Glass slides were immersed in a 5% (v/v) solution of Decon-90 (a commercially available detergent) for 2 h. They were then rinsed with copious amounts of deionised water and cleaned in an ultrasonic bath twice, each time for 15 min. Finally the surfaces of the glass slides were rinsed thoroughly with deionised water.

For the preparation of PEI-coated glass slides, clean slides were immersed in an aqueous solution containing 1% (w/v) PEI for 30 min at room temperature. The slides were then rinsed thoroughly with deionised water and dried under a stream of nitrogen gas. The PEI-coated glass slides were rubbed firmly 8–9 times in the same direction with a velvet cloth prior to use.

For the preparation of DMOAP-coated glass slides, clean slides were immersed in an aqueous solution containing 0.1% (v/v) DMOAP for 5 min at room temperature. The slides were then rinsed with copious amounts of deionised water, dried under a stream of nitrogen gas and heated in a vacuum oven at 100°C for 15 min.

2.4 *Preparation of* E. coli-*decorated surfaces*

Droplets (~2 μL) of buffer solutions containing different *E. coli* strains were spotted on the surfaces of PEI-coated glass slides in an array format. This surface was then incubated in a humid chamber to avoid evaporation of water droplets. After 30 min incubation at room temperature, the surface was washed with buffer and deionised water, and then dried under a stream of nitrogen gas.

To prepare the samples used in atomic force microscopy (AFM) and optical microscopy characterisation, 200 μL of buffer solutions containing different *E. coli* strains were applied to the surfaces of PEI-coated glass slides. After 30 min of incubation at room temperature, the surface was rinsed with buffer solution and deionised water. The sample was then dried under a stream of nitrogen.

2.5 *Fabrication of LC optical cells*

An LC optical cell was fabricated by sandwiching two glass slides (a DMOAP-coated slide and an *E. coli*-decorated slide) separated by two strips of spacer (~6 μm) at both ends of the slide. The optical cell was secured with two binder clips. 5CB was then drawn into the cavity formed between the two glass surfaces through capillary force [18]. The optical textures of the 5CB inside the optical cell were observed using a polarising microscope (Nikon ECLIPSE LV100POL, Japan) in the transmission mode. Each image was captured by a digital camera (Nikon DS-U1, Japan) mounted on the microscope with an exposure time of 25 ms.

2.6 *X-ray photoelectron spectroscopy (XPS)*

All XPS data were obtained following a previously reported procedure [18]. The instrument was an ultra-high vacuum XPS system AXIS HIS (Kratos, UK) equipped with a monochromotised Al Kα X-ray source (1486.6 eV) at a constant dwell time of 100 ms and a pass energy of 40 eV. All binding energies were referenced to the C1s hydrocarbon peak at 284.6 eV.

2.7 *Atomic force microscopy*

Tapping mode AFM measurement was performed using a Multi–mode SPM with controller III_a (Veeco Instruments, USA) with a high-aspect ratio tapping tip. A scanning rate of around 0.3 Hz was used.

2.8 *Optical microscopy*

The surface density of *E. coli* was obtained using an optical microscope (Eclipse E200, Nikon, Japan) with a digital camera (Nikon DS-U1, Japan) mounted on the top. The total number of *E. coli* in each image was counted manually. The surface density of bound *E. coli* was calculated by dividing the total number of *E. coli* in each image by the total image area (40 μm × 40 μm).

3. Results and discussion

3.1 *Attraction between PEI surface and* E. coli

As a Gram-negative bacterium, the extracellular membrane of *E. coli* consists of approximately 75% lipopolysaccharides (LPS) and 25% proteins as the main components [19]. Therefore, *E. coli* has negative charge in the solution at pH > 4 and can easily adsorb onto positively charged surfaces [20–23]. Therefore, poly (ethylene imine) (PEI) was chosen to modify the glass surfaces because PEI is a polycationic polymer which is known to attract negatively charged *E. coli* [24].

To find out under what experimental conditions a PEI-coated glass slide is able to promote the adsorption of negatively charged *E. coli* onto the glass surface, we immersed clean slides into PEI solutions of various concentrations for 30 min at 24°C. Since the clean glass surface has negative charge, PEI can easily form a uniform layer on the surface [25]. After the immersion, the glass surfaces were rinsed thoroughly with water and dried under a stream of nitrogen gas.

The presence and the quantity of the amine functional groups deposited on the surface were analysed using X-ray photoelectron spectroscopy (XPS). From Table 1, it can be seen that the untreated glass (0% PEI) has 3.52% of nitrogen (N) on the surface while the glass treated with 0.1% (w/v) PEI solution has 17.85% N. When the PEI concentration is 5% (w/v), the N content also increases to 23.63%. These results indicate that PEI was successfully deposited onto the glass surface and that the surface density of PEI can be controlled by varying the PEI concentration.

To investigate the efficiency of PEI-treated surfaces in attracting *E. coli*, various PEI-coated glass slides were incubated in 200 μL of PBS containing *E. coli* TOP10 strain which was harvested from an agar plate and diluted to an optical density (OD) of 0.2. After incubation for 30 min at 24°C, the glass slides were rinsed with PBS and deionised water.

Table 1. XPS surface chemical composition of glass slides after being treated with various concentrations of PEI.

	N	C
Glass	3.52	96.48
0.1% PEI	17.85	82.15
0.5% PEI	21.55	78.45
1% PEI	21.79	78.21
5% PEI	23.63	76.37

The amount of *E. coli* TOP10 adsorbed on the glass surface was probed using both atomic force microscopy (AFM) and optical microscopy (Figure 1). *E. coli* is a rod-like bacterium with a length of 1–2 μm and a diameter of 800 nm. Figure 1(a) shows no *E. coli* on the clean glass (0% PEI). On the contrary, *E. coli* can be found on PEI-coated surfaces and the amount of *E. coli* on the surfaces increases with the PEI concentrations (Figure 1(b)-(e)) except when the PEI concentration is 5%.

Both AFM and optical microscopy indicates that the 5% PEI-coated surface (Figure 1(e)) does not adsorb *E. coli* more than the one coated with 1% PEI solution (Figure 1(d)). The AFM image also indicates that the surface of the 5% PEI-coated surface is not very uniform. Past studies have revealed that 5% PEI may form thicker layers on the surface. Thus, some PEI layers might be rinsed away together with *E. coli* [26, 27] resulting in a lower density of *E. coli* on the surface, which leads to unsatisfied absorption of *E. coli* on the 5% PEI-coated surface.

The density of bound *E. coli* was also quantified based on microscopic images (Figure 1). The density is 5.19×10^4 cell/mm^2 on the 0.1% PEI-coated surface, increasing to 5.43×10^4/mm^2 when PEI concentration is 0.5%. The 1% PEI-coated surface has the highest *E. coli* density (up to 6.44×10^4 cell/mm^2), while 5% PEI-coated surface has only 3.69×10^4 cell/mm^2. These results suggested that a 1% PEI solution would be sufficient to produce a PEI layer on the glass surface for the adsorption of *E. coli* in the subsequent experiments.

Next, we investigated the optical textures and orientational behaviours of a nematic LC, 5CB, on PEI-coated surfaces with surface-bound *E. coli*. Because the surface charge of *E. coli* depends on pH, the effect of pH was also studied. *E. coli* TOP10 was harvested from agar plates and dispersed in 10 mM of various buffers. The LC optical cell was then

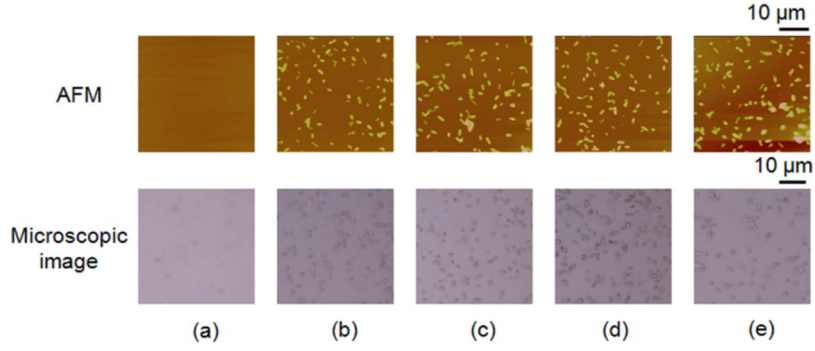

Figure 1. AFM (top) and microscopic (bottom) images of glass slides after being incubated with *E. coli* TOP10 in PBS buffer solutions for 30 min. Prior to incubation, the slides were treated with PEI at concentrations of (a) 0%, (b) 0.1%, (c) 0.5%, (d) 1% and (e) 5%. The corresponding surface densities of *E. coli* on these images are (a) 0 cell/mm^2, (b) 5.19×10^4 cell/mm^2, (c) 5.44×10^4 cell/mm^2, (d) 6.44×10^4 cell/mm^2 and (e) 3.69×10^4 cell/mm^2 (colour version online).

fabricated by pairing an *E. coli*-spotted surface and a glass slide treated with N, N-dimethyl-*n*-octadecyl-3-aminopropyl trimethoxysilyl chloride (DMOAP).

Figure 2 shows images of 5CB (under crossed polars) in the optical cells mentioned above. The circular regions are where droplets of buffer containing *E. coli* TOP10 were dispensed (OD = 0.2). Figure 2(a)–(d) shows that the optical appearance of 5CB on the PEI-coated surface (outside circle regions) is uniformly bright, indicating a near planar azimuthal orientation of 5CB on the surface. However, the optical appearance of 5CB in the circular region of Figure 2(b) is always dark upon rotating the sample under crossed polars for 360°. This phenomenon indicates homeotropic (perpendicular) orientations of 5CB in the cell. Also note in Figure 2(c) that, although the optical appearance of 5CB in the circular region is not completely dark, it is still spatially distinguishable from the uniform bright background, indicating different azimuthal and polar orientations of 5CB in the circular region. In contrast, when the PEI-coated surface was spotted with *E. coli* TOP10 dispersed in either acidic buffer (pH 4) or basic buffer (pH 10), the optical appearance of 5CB was fully bright (Figure 2(a) and (d), circular region). The bright domains in these circular regions could still be recognised under the bright background, but they are not as clear as those in Figure 2(b) and 2(c). Furthermore, AFM and microscopic images of these samples show that the amount of *E. coli* adsorbed on the surface at pH 4 and pH 10 is much less than that at pH 6 and pH 8.

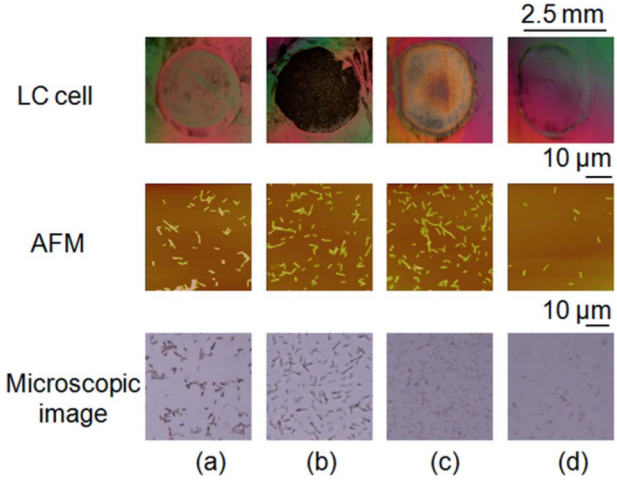

Figure 2. Cross-polarised LC (top), AFM (middle) and microscopic (bottom) images of glass surfaces after being incubated with *E. coli* TOP10 at a pH of (a) 4, (b) 6, (c) 8 and (d) 10. The corresponding surface densities of *E. coli* on these images are (a) 5.75×10^4 cell/mm^2, (b) 7.25×10^4 cell/mm^2, (c) 10.0×10^4 cell/mm^2 and (d) 2.81×10^4 cell/mm^2.

Previous studies have shown that *E. coli* becomes less negatively charged in acidic solutions and that amine functional groups are less protonated at pH = 10 [20–23, 28]. Both situations can reduce the electrostatic attraction between *E. coli* and the aminated surface, leading to less adsorbed *E. coli* on the surface.

These results, when combined, suggest that pH 6–8 is the optimum pH for detecting *E. coli* using the PEI-coated slide and LCs. Accordingly, PBS (pH 7.4) was used in the following experiments.

3.2 *Selectivity of different strains*

To further investigate LC responses to different *E. coli* strains, similar experiments were conducted using two additional *E. coli* strains, DH5α and JM109. Figure 3(a)–(c) shows the comparison of LC, AFM and microscopic images obtained by using three *E. coli* strains (TOP10, DH5α and JM109). Both the AFM and microscopic images indicate no difference in the *E. coli* surface density for all three strains used. However, in Figure 3(a), the optical appearance of 5CB in the region with *E. coli* TOP10 is uniformly dark, but in Figure 3(b) and 3(c), the optical appearances of 5CB in the circular regions corresponding to DH5α and JM109 are bright. The results obtained reveal that only *E. coli* TOP10 strain, and not strains DH5α or JM109, has the ability to induce a homeotropic orientation of 5CB at the surface and cause a dark image.

To determine whether the changes in the orientations of 5CB are caused by the EPS presenting on the surface of *E. coli*, we performed similar experiments except that *E. coli* was grown in LB broth rather than on agar plates. This is because past studies have shown that *E. coli* grown in LB broth and purified via

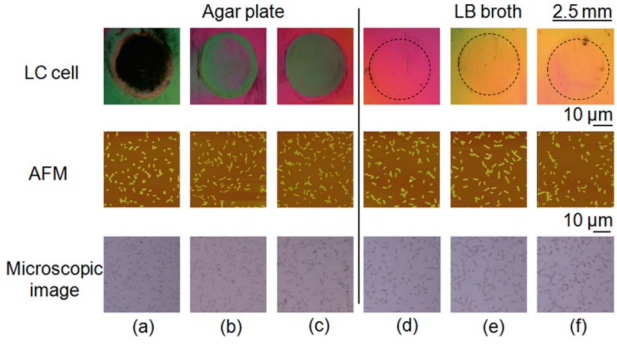

Figure 3. Cross-polarised LC (top), AFM (middle) and microscopic (bottom) images of 1% PEI-treated glass surfaces after being incubated with different *E. coli* strains (a) TOP10, (b) DH5α and (c) JM109 grown on agar plates, (d) TOP10, (e) DH5α and (f) JM109 grown in LB broth. The corresponding surface densities of *E. coli* on these images are (a) 7.19×10^4 cell/mm^2, (b) 7.12×10^4 cell/mm^2, (c) 7.25×10^4 cell/mm^2, (d) 6.69×10^4 cell/mm^2, (e) 7.31×10^4 cell/mm^2 and (f) 7.75×10^4 cell/mm^2.

centrifugation and washing has much less EPS on its surface than that grown on agar plates [29–32].

Both AFM and microscopic images revealed no difference (in terms of surface density of *E. coli*) for *E. coli* grown in LB broth (Figure 3(d)–f)) or grown on agar plates (Figure 3(a)–(c)), suggesting that the medium in which the *E. coli* grow does not affect its adsorption on the PEI-coated surface. However, we found that none of the *E. coli* strains grown in LB broth induced any measurable changes in the LC (Figure 3(d)–(f), top), and that neither the growth media nor the buffers (control experiments) affected the LC appearance on the PEI surface (data not shown).

Thus, the changes in the orientations of LCs on the surfaces onto which *E. coli* was spotted are probably due to the EPS presenting on *E. coli* surfaces. Since different bacterial species have different genes which determine the amount and composition of EPS produced, the LC-based detection method described here may give different visual responses to different strains of bacteria, and can be applied for rapid and simple detection and speciation of different bacteria strains [33].

3.3 *Sensitivity of* E. coli *TOP10*

Finally, we investigated the effect of the concentrations of *E. coli* TOP10 strain (grown on agar plates) on LC responses to estimate the minimal *E. coli* surface density that can cause homeotropic or tilted orientation of the LCs. We prepared PBS solutions containing six different *E. coli* TOP10 concentrations (OD = 0.05, 0.10, 0.15, 0.20, 0.25, and 0.30, respectively), and dispensed droplets of these solutions onto PEI-coated slides.

Figure 4(a)–(f) show optical images of 5CB made from these slides. All spots contacted with lower concentrations of *E. coli* TOP10 (OD $<$ 0.2) have fully bright optical textures (Figure 4(a)–(c)). However, when the OD is $>$0.2, the optical textures of 5CB became dark or are a mixture of dark and bright (Figure 4(d)–(f)). Microscopic and AFM images confirm that the amount of *E. coli* adsorbed on the PEI-coated surface increases with increasing OD, but that there is no change in the orientation of 5CB before the density of *E. coli* reaches a critical concentration (around 6×10^4 cell/mm^2).

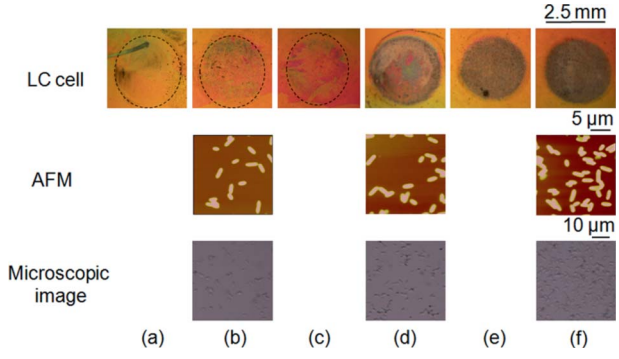

Figure 4. Optical cross-polarised LC (top), AFM (middle) and microscopic (bottom) images of 1% PEI-treated glass surfaces after being incubated with *E. coli* TOP10 at concentrations of (a) OD = 0.05, (b) OD = 0.10, (c) OD = 0.15, (d) OD = 0.20, (e) OD = 0.25 and (f) OD = 0.30. The corresponding surface densities of *E. coli* on these images are (b) 2.81×10^4 cell/mm^2, (d) 6.50×10^4 cell/mm^2, (f) 9.50×10^4 cell/mm^2 (colour version online).

4. Conclusions

We have demonstrated that PEI-coated surfaces are capable of capturing *E. coli* through electrostatic attraction at neutral pH. Only *E. coli* TOP10 grown on agar plates can disrupt orientations of LCs; DH5α and JM109 cannot. The difference gives a clear distinction in the optical appearance of LCs as either dark or bright images. We also found that only *E. coli* TOP10 grown on agar plates (and not in LB broth) can change the orientations of LCs, suggesting that these orientational changes are probably due to the EPS produced on the *E. coli* surface.

The LC-based bacteria identification method reported here may provide a simple, rapid and low cost means to identify certain *E. coli* strains without a requirement for complex instrumentation and labelling agents.

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References

[1] Gupta, V.K.; Skaife, J.J.; Dubrovsky, T.B.; Abbott, N.L. *Science* **1998**, *279*, 2077–2080.
[2] Woltman, S.J.; Jay, G.D.; Crawford, G.P. *Nat. Mater.* **2007**, *6*, 929–938.
[3] Kim, S.R.; Abbott, N.L. *Adv. Mater.* **2001**, *13*, 1445–1449.
[4] Kim, S.R.; Abbott, N.L. *Langmuir* **2002**, *18*, 5269–5276.
[5] Luk, Y.Y.; Tingey, M.L.; Dickson, K.A.; Raines, R.T.; Abbott, N.L. *J. Am. Chem. Soc.* **2004**, *126*, 9024–9032.
[6] Xue, C.Y.; Khan, S.A.; Yang, K.-L. *Adv. Mater.* **2009**, *21*, 198–202.
[7] Lai, S.L.; Huang, S.S.; Bi, X.Y.; Yang, K.-L. *Langmuir* **2009**, *25*, 311–316.

[8] Price, D.; Schwatz, D.K. *J. Am. Chem. Soc*. **2008**, *130*, 8188–8194.
[9] Brake, J.M.; Daschner, M.K.; Luk, Y.Y.; Abbott, N.L. *Science* **2003**, *302*, 2094–2097.
[10] Hartono, D.; Bi, X.Y.; Yang, K.-L.; Yung, L.-Y.L. *Adv. Funct. Mater*. **2008**, *18*, 2938–2945.
[11] Fang, J.; Ma, W.; Selinger, J.V.; Shashidhar, R. *Langmuir* **2003**, *19*, 2865–2869.
[12] Luk, Y.Y.; Campbell, S.F.; Abbott, N.L. Murphy, C.J. *Liq. Cryst*. **2004**, *31*, 611–621.
[13] Lockwood, N.A.; Mohr, J.C.; Ji, L.; Murphy, C.J.; Palecek, S.P.; de Pablo, J.J.; Abbott, N.L. *Adv. Funct. Mater*. **2006**, *16*, 618–624.
[14] Jang, C.H.; Cheng, L.L.; Olsen, C.W.; Abbott, N.L. *Nano Lett*. **2006**, *6*, 1053–1058.
[15] Tercero Espinoza, L.A.; Schumann, K.R.; Luk, Y.Y.; Israel, B.A.; Abbott, N.L. *Langmuir* **2004**, *20*, 2375–2385.
[16] Helfinstine, S.L.; Lavrentovich, O.D.; Woolverton, C.J. *Lett. Appl. Microbiol*. **2006**, *43*, 27–32.
[17] Sivakumar, S.; Wark, K.L.; Gupta, J.K.; Abbott, N.L.; Caruso, F. *Adv. Funct. Mater*. **2009**, *19*, 2260–2265.
[18] Xu, H.; Bi, X.Y.; Ngo, X.M.; Yang, K.-L. *Analyst* **2009**, *134*, 911–915.
[19] Amro, N.; Kotra, L.; Wadu-Mesthrige, K.; Bulychev, A.; Mobashery, S.; Liu, G. *Langmuir* **2000**, *16*, 2789–2796.
[20] Terada, A.; Yuasa, A.; Kushimoto, T.; Tsuneda, S.; Katakai, A.; Tamada, M. *Microbiology* **2006**, *152*, 3575–3583.
[21] Blake, D.M.; Maness, P.C.; Huang, Z.; Wolfrum, E.J.; Huang, J.; Jacoby, W.A. *Sep. Purif. Rev*. **1999**, *81*, 1–50.
[22] Lin, D.Q.; Zhong, L.N.; Yao, S. *Biotechnol. Bioeng*. **2006**, *95*, 186–191.
[23] Lin, D.Q.; Brixius, P.J.; Hubbuch, J.J.; Thömmes, J.; Kula, M.R. *Biotechnol. Bioeng*. **2003**, *83*, 149–157.
[24] Chu, Y.F.; Hsu, C.H.; Soma, P.K.; Lo, Y.M. *Bioresour. Technol*. **2009**, *100*, 3167–3174.
[25] Plech, A.; Salditt, T.; Münster, C.; Peisl, J. *J. Colloid Interface Sci*. **2000**, *223*, 74–82.
[26] Bai, Y.; Huang, W.C.; Yang, S.T. *Biotechnol. Bioeng*. **2007**, *98*, 328–339.
[27] Lin, Y.; Huck, W.T.S.H.; Zhao, X.M.; Whitesides, G.M. *Langmuir* **1999**, *15*, 1208–1214.
[28] Sonohara, R.; Muramatsu, M.; Ohshima, H.; Kondo, T. *Biophys. Chem*. **1995**, *55*, 273–277.
[29] Sheng, G.P.; Yu, H.Q.; Yu, Z. *Appl. Microbiol. Biotechnol*. **2005**, *67*, 125–130.
[30] Nielsen, P.H.; Jahn, A.; Palmgren, R. *Water Sci. Technol*. **1997**, *9*, 11–19.
[31] Zhang, X.Q.; Bishop, P.L.; Kinkle, B.K. *Water Sci. Technol*. **1999**, *39*, 211–218.
[32] Wolfstein, K.; Stal, L.J. *Mar. Ecol. Prog. Ser*. **2002**, *236*, 13–22.
[33] Nierop Groot, M.N.; Kleerebezem, M. *J. Appl. Microbiol*. **2007**, *103*, 2645–2656.