Biological treatment of refinery spent caustics under halo-alkaline conditions

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A B S T R A C T

The present research demonstrates the biological treatment of refinery sulfidic spent caustics in a continuously fed system under halo-alkaline conditions (i.e. pH 9.5; Na+ = 0.8 M). Experiments were performed in identical gas-lift bioreactors operated under aerobic conditions (80–90% saturation) at 35 °C. Sulfide loading rates up to 27 mmol L−1 day−1 were successfully applied at a HRT of 3.5 days. Sulfide was completely converted into sulfate by the haloalkaliphilic sulfide-oxidizing bacteria belonging to the genus Thioalkalivibrio. Influent benzene concentrations ranged from 100 to 600 μM. At steady state, benzene was removed by 93% due to high stripping efficiencies and biodegradation. Microbial community analysis revealed the presence of haloalkaliphilic heterotrophic bacteria belonging to the genera Marinobacter, Halomonas and Idiomarina which might have been involved in the observed benzene removal. The work shows the potential of halo-alkaliphilic bacteria in mitigating environmental problems caused by alkaline waste.

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1. Introduction

Diluted caustic (NaOH) solutions are often used in the petrochemical industry for the removal of acidic compounds, e.g. volatile (organic) sulfur compounds from hydrocarbon streams, such as gaseous streams and LPG. The use of caustic leads to the formation of a waste product referred to as sulfidic spent caustic. These spent caustic solutions originating from oil refineries are characterized by a high pH (pH > 12) and elevated sodium concentrations up to 5–12% by weight (Alnaizy, 2008). Total dissolved sulfide (i.e. the sum of H2S, S2−/C0 and S2−/C0) typically are the most dominant sulfur compounds found in spent caustics with concentrations that may exceed 2–3 wt% (Conner et al., 2000). Total dissolved sulfide (i.e. the sum of H2S, HS− and S2−) is well known for its toxic, odorous and corrosive properties. Besides total dissolved sulfide, a variety of organic sulfur compounds and aromatic hydrocarbon compounds are commonly found in spent caustics of which methanethiol (CH3SH), benzene, toluene and phenol are most pronounced (Alnaizy, 2008; Olmos et al., 2004; Park et al., 2010; Sipma et al., 2004).

The actual composition of spent caustics is, however, very much dependent on the type of hydrocarbon stream that has been treated.

Due to more stringent (environmental) regulations, the transport and handling costs of spent caustics are currently very high. Disposal of spent caustics for either reuse or product recovery purposes is therefore becoming less economically attractive. In addition, the fluctuations in caustic quality, due to differences in the crude oil composition and storage policies at different refineries, causes problems for the companies that process spent caustics (Alnaizy, 2008).

Wet air oxidation is generally applied for the physico-chemical treatment of spent caustics. In this process, soluble or suspended compounds are partially or completely oxidized at elevated temperatures and pressures using air-oxygen as the oxidizing agent (Ellis, 1998). Chemical treatment of sulfidic spent caustics, such as treatment with hydrogen-peroxide, most often leads to an incomplete oxidation of the dissolved sulfide to thiosulfate and hence in a residual chemical oxygen demand of the treated water. Moreover, the storage and handling of the hydrogen peroxide is associated with considerable safety measures.

Biological treatment of spent caustics, at atmospheric pressures and temperatures would be a cheaper and safer alternative to the currently employed physico-chemical treatment processes. On-site treatment of spent caustics in conventional biological waste water treatment plants is standard practice at many refineries. Although addition of small amounts of spent caustics to a biotreater can
work to some extent, these processes are not designed to handle large amounts of complex spent caustic waste streams as the biological processes can easily be disturbed by fluctuating pH conditions, increasing salt concentrations and the accumulation of toxic compounds (Metcalfe and Eddy, 1991). Typical spent caustic production rates may amount up to 15 m³/day (Olmos et al., 2004). This represents a significant portion of the hydraulic and COD load to the refineries biological wastewater treatment plant (Pinzón Pardo et al., 2007). Moreover, the growth of filamentous bacteria, such as sulfide-oxidizing Thiothrix species in activated sludge systems may lead to severe operating problems as a result of the formation of bulking sludge (Nielsen, 1985). Previous research has shown that dilution factors up to three had to be applied in order to lower the pH and sodium levels down to acceptable concentrations for neutrophilic sulfide-oxidizing bacteria (SOB) (Sipma et al., 2004). Particularly in arid regions this would be a serious drawback for the application of this new process. Hence, investigation of the application of haloalkaliphilic microorganisms for the treatment of complex sulfide-containing waste streams is of great interest. Recently, a new biotechnological process has been described for the removal of hydrogen sulfide from high-pressure natural gas (Van den Bosch et al., 2007). This process relies on a specialized group of haloalkaliphilic sulfide oxidizing bacteria that is also considered for the treatment of undiluted sulfidic spent caustics (Sorokin and Kuenen, 2005).

In the current study, attention is also paid to the fate of benzene because it is well known for its relatively high water solubility, stability and carcinogenic properties (Sikkema et al., 1995). Hence, it has to be removed from spent caustic solutions prior to discharge into the environment. Up till now a limited amount of literature is available on the biodegradation of mono-aromatics under haloalkaline conditions (Alva and Peyton, 2003; Le Borgne et al., 2008; Margesin and Schinner, 2001). Furthermore, the effects of benzene on biological sulfide oxidation have not been investigated. Two bioreactors were inoculated with biomass obtained from soda lake sediments and continuously fed with spent caustic solutions collected from a refinery. In two long-term test runs, the oxidation of sulfide into sulfate and the removal of benzene has been evaluated over periods of 78 and 55 days, respectively. Denaturing gradient gel electrophoresis (DGGE) and cloning of PCR-amplified 16S rRNA gene fragments were used to monitor the microbial community dynamics during the experimental period to identify the community members.

2. Methods

2.1. Experimental set-up

Two continuously operated gas-lift reactors with a liquid volume of 2.2 L (⌀ = 10 cm) were used (Fig. 1). Temperature was maintained constant at 35 ºC using a water-jacket and a thermostat bath (Haake, Germany). Influent was added to the reactor using peristaltic pumps (Masterflex® L/S®, Cole-Parmer instruments, USA). The influent was added to the downer section of the reactor to prevent short-circuiting. pH was monitored using a pH sensor (Endress+Hauser orbisint CPS12D, Naarden, The Netherlands).

Conductivity (mS cm⁻¹) was measured using a conductivity meter (Masterflex® L/S®, Cole-Parmer instruments, USA). The percentage of oxygen saturation (% sat) was monitored (Mettler Toledo Inpro 6050 oxygen sensor) and controlled at 80–90% by supplying pure oxygen via mass flow controllers (Bronkhorst, The Netherlands). The gas phase was continuously recycled using a small compressor (NB20, 20 L min⁻¹), KNF pumps, Germany) (Fig. 1 A). The recirculation gas phase first passes a condenser (10 ºC) to recover volatile compounds that are stripped from the bioreactor suspension (Fig. 1 B).

2.2. Inoculum and influent

The inoculum consisted of Russian soda lakes sediments (Kulunda Steppe, Altai) that were kindly provided by Delft University of Technology (Sorokin et al., 2010).

Two spent caustic solutions (Solution A and B), taken from the same refinery at different moments in time, were used as influent solutions (Table 1). Because the sulfide concentrations in Solution A and B were very high, the solutions were mixed with sodium carbonate (Na₂CO₃) solutions that were prepared at the same salinity (0.8 M) and pH (pH 9.5). This improved the stability of the system by decreasing the hydraulic retention time (HRT) whilst keeping the sulfide load and sodium concentration in the influent constant. During the experimental runs, the ratio of spent caustic solution over Na₂CO₃ solution was changed to meet the desired HRT. NaCl (1 g L⁻¹) was added to the Na₂CO₃ solution to meet the chloride requirements for growth of haloalkaliphilic sulfide oxidizing bacteria (Sorokin et al., 2010).

Table 2. Characterization of raw refinery spent caustic solutions, sampled at two different moments in time.

<table>
<thead>
<tr>
<th></th>
<th>Solution A</th>
<th>Solution B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfide (mM)</td>
<td>240</td>
<td>160</td>
</tr>
<tr>
<td>Benzene (mM)</td>
<td>0.6</td>
<td>10</td>
</tr>
<tr>
<td>Sodium (M)</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Conductivity (mS cm⁻¹)</td>
<td>92</td>
<td>94</td>
</tr>
<tr>
<td>pH</td>
<td>13.2</td>
<td>13</td>
</tr>
</tbody>
</table>

Fig. 1. Schematic overview of the continuously Run gas-lift reactors. Sensoring and controlling units for oxygen, pH and redox are shown as well as the gas recirculation pump (A), condenser (B), and pH (C) and oxygen (D) controlling unit. Bleed gas left the reactor via a series of washing bottles.
Table 2
Influent sulfide and benzene concentrations during different periods of Run 1 and 2. 
Sulfide concentrations for Run 2 were increased to a concentration of 61 mM. In addition, the specific spent caustic solution that was used for each Run and the mixing ratio of spent caustic with the sodium carbonate (0.8 M; pH = 9.5) solution are given.

<table>
<thead>
<tr>
<th>Period</th>
<th>Run 1</th>
<th>Run 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Time (days)</td>
<td>0–34</td>
<td>34–55</td>
</tr>
<tr>
<td>Spent caustic</td>
<td>Solution A</td>
<td></td>
</tr>
<tr>
<td>Spent caustic/soda mixing ratio</td>
<td>1</td>
<td>6.2</td>
</tr>
<tr>
<td>Sulfide (μM)</td>
<td>240 ± 2</td>
<td>39 ± 7</td>
</tr>
<tr>
<td>Benzene (μM)</td>
<td>600 ± 6</td>
<td>100 ± 9</td>
</tr>
</tbody>
</table>

bacteria (Sorokin and Kuenen, 2005). Macro-nutrients (range of 2.5–4×2.2 m l min⁻¹ for HRT 30–3.5 days) were continuously added to the reactor liquid in the following amounts: 1 g l⁻¹ K₂HPO₄, 0.2 g l⁻¹ MgCl₂·6 H₂O and 0.6 g l⁻¹ urea. Trace element solution (0.5 ml l⁻¹) was added as described elsewhere (Pfenning and Lippert, 1966).

2.3. Experimental design

Run 1 and 2 were performed in separate reactor systems. Operating conditions of both runs are given in Table 2. During start-up (period I) of Run 1, the pure spent caustic (Solution A) was added to the system. After start-up period the spent caustic solution A was mixed with the Na₂CO₃ solution to obtain the desired HRT and sulfide load. At day 34, the pH was decreased from 10.4 to 9.8 by adding CO₂. The biological sulfide oxidation system was operated in a constant flow mode with helium as a carrier gas (1.0 ml min⁻¹). The oven temperatures were operated at 40 °C for 2 min, 15 °C min⁻¹ ramp to 100 °C, 50 °C min⁻¹ ramp to 200 °C. The MS (source temperature = 230 °C and quadrupole temperature = 150 °C) was operated in Select Ion Mode (SIM). Headspace vials (10 ml) were filled with 5 ml of liquid sample and an excess (3 g) of sodium chloride. External standards were prepared in ethanol and diluted in water. Benzene-d₆ was used as internal standard and was obtained from Sigma–Aldrich (The Netherlands). The headspace vials were equilibrated for 15 min at a temperature of 70 °C. The probe temperature was set to 80 °C and the transfer line temperature to 90 °C. The headspace of the vial was pressurized to 1 bar overpressure prior to injection. A 2.5 ml gas sample was injected into the GC-MS.

2.5. Microbial community analysis

Genomic DNA was extracted from reactor samples of Run 1 using the FastDNA SPIN soil kit (MP Biomedicals, USA).

For denaturing gradient gel electrophoresis (DGGE) analysis, partial 16S rDNA was amplified using the bacterial primers GC341f and 907rM. DGGE was performed as described by Schäfer and Muyzer (2001) using a denaturing gradient of 30–60% denaturants (urea and formamide; UF) in 8% polyacrylamide gel. Individual bands were excised, placed in 10 mM Tris buffer, re-amplified and sequenced.

The nearly complete 16S rDNA of samples taken on day 7, 39 and 75 of Run 1 were amplified using bacterial primers GM3f and GM4r (Brinkhoff et al., 1998). The PCR products were ligated into pCR4-TOPO and transformed into competent cells of Escherichia coli according to the TA Cloning kit (Invitrogen, USA). Transformed cells were plated on Luria–Bertani medium plates containing 50 μg ml⁻¹ kanamycin. After overnight incubation at 35 °C, clones were randomly selected for sequencing. PCR products for sequencing were purified using the Qiapquick PCR purification kit (Qiagen) and sequenced by a commercial company (Macrogen, South Korea).

The obtained 16S rRNA gene sequences were first compared to sequences stored in GenBank using the BLASTN algorithm (Altschul et al., 1990). Subsequently, the sequences were aligned using the SILVA website, imported into ARB and added to a neighbor-joining tree made of complete sequences. The sequences have been stored in GenBank under accession numbers: HQ413781–HQ414030.

3. Results and discussion

3.1. Biological sulfide oxidation

From Fig. 2A and C it can be seen that sulfide was (almost) completely removed during Run 1 and 2; sulfide effluent concentrations were below the detection limit of 3 μg l⁻¹ (data not shown). Hence, sulfide removal from spent caustics has been proven to be successful for influent sulfide concentrations up to...
90 mM and loading rates up to 27 mmol sulfide L$^{-1}$ day$^{-1}$ (Run 1, period III). Sulfide was converted into sulfuric acid (H$_2$SO$_4$) resulting in a small pH decrease. When using a raw refinery spent caustic as a feedstock, it is likely that the produced H$_2$SO$_4$ can replace chemicals for pH control resulting in lower overall operating costs.

Sulfide conversion into sulfate (sulfate selectivity) amounted up to 88 mol% during steady state (day 68–78) of Run 1 (Table 3). Little sulfur formation was observed as no whitish colloidal particles could be seen in the bioreactor. Therefore, quantification of elemental sulfur was not attempted. Moreover, in the event that small amounts of elemental sulfur would be formed this is difficult to quantify due to attachment of the sulfur particles onto the reactor wall. No thiosulfate was formed during this period of Run 1, meaning that any abiotic sulfide oxidation did not occur (Janssen et al., 1995).

From Fig. 2C, it follows that more than 90% of the sulfide was converted into sulfate during period I of Run 2 (day 8–20) at a HRT of 30 days. During steady state of Run 2 (day 41–57), sulfate selectivity amounted up to 96 mol% (Table 3). Hence, it can be concluded that during this run an almost complete oxidation to sulfate was achieved (Fig. 2C). The small difference in the sulfur balance can be attributed to losses as a result of assimilation processes and perhaps some analytical inaccuracies. In Run 2, the thiosulfate concentration was always below the detection limit of 2 $\mu$M (Fig. 2C).

At day 22 (Run 2), the sulfate concentration in the reactor was decreased because half of the reactor fluid was replaced with fresh buffer solution (Fig. 2C).

### Table 3

Average influent concentrations, sulfide load and sulfate selectivity during steady state in period III of Run 1 and 2.

<table>
<thead>
<tr>
<th></th>
<th>Run 1</th>
<th>Run 2</th>
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</thead>
<tbody>
<tr>
<td>Time (days)</td>
<td>68–78</td>
<td>41–57</td>
</tr>
<tr>
<td>HRT (days)</td>
<td>3.4</td>
<td>3.7</td>
</tr>
<tr>
<td>Influent sulfide (mM)</td>
<td>93 ± 3</td>
<td>61 ± 2</td>
</tr>
<tr>
<td>Sulfide load (mmol L$^{-1}$ day$^{-1}$)</td>
<td>27 ± 1</td>
<td>16 ± 1</td>
</tr>
<tr>
<td>$\text{SO}_4^{2-}$ selectivity (mol%)</td>
<td>88 ± 2</td>
<td>96 ± 4</td>
</tr>
</tbody>
</table>

3.2. **Biomass concentration**

Biomass concentrations roughly fluctuated around 6–8 mg N L$^{-1}$ for Run 1 (Fig. 2B) while showing an increasing trend up to day 52 (13 mg N L$^{-1}$) for Run 2 (Fig. 2D). The biomass was able to grow and thrive at the employed operating conditions without the use of a biomass retention system such as a settler, carrier material or a membrane. In case of biomass retention, the number of organisms in the reactor will increase, resulting in higher treatment capacities. This could be beneficial to a full-scale industrial application since this leads to smaller reactor sizes and thus lower investment costs.

The observed decrease in biomass during period I (Run 1, day 0–34) may be attributed to the wash out of non-sulfide oxidizing microorganisms that were present in the initial inoculum (Fig. 2B). At day 34 of Run 1, a sulfate selectivity of 80 mol% was achieved (Fig. 2B). In period I, a pure (i.e. non-mixed) spent caustic (Solution A) was used as influent with a sulfide concentration of 0.24 M at a HRT of 32.5 days (Fig. 2A). From the whitish
appearance of the reactor suspension during period I, it can be con-
cluded that a fraction of the sulfide was oxidized to elemental sul-
fur. This process is accompanied by the formation of hydroxide
which caused the pH to increase from 9.8 to 10.4 between day 0
and 34. Thiocyanate concentrations up to 40 mM were recorded
from day 0–11 (Fig. 2A). As a result of O₂-limiting conditions due
to failure of the O₂-sensor, the accumulation of thiocyanate can be
attributed to the chemical, i.e. non-biological, oxidation of sulfide
(Janssen et al., 1995). Immediately after replacement of the O₂-sen-
sor and adjustment of the O₂ supply, the sulfide oxidation capacity
was fully restored. Moreover, the formed thiocyanate was converted
to sulfate reaching concentrations less than 2 μM.

The increasing biomass concentrations observed in Run 2 (day 41–52) resulted from increased sulfide loading rates (Fig. 2D).

### 3.3. Benzene removal efficiency

Table 4 shows the benzene removal at steady state conditions of the biological experiments (Run 1 and 2) and the abiotic control experiment. Average benzene removal efficiencies of 93% were found during final steady state of Run 1 and 2 (Table 4).

The control experiment shows that in the absence of microor-
ganisms 67% of the benzene was stripped from the gas-lift reactor
liquid (Table 4). The benzene was continuously removed from the reactor liquid to the gaseous phase and then left the system via the bleed gas stream (Fig. 1). The ability to remove benzene by strip-
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in the absence of biomass (Table 4). The benzene was continuously removed from the reactor run (Fig. 3). A shift in dominance between different phyl-
otypes of *Thioalkalivibrio* could be the result of small changes in the operating conditions and might indicate subtle differentiations
within the same niche (genetic microdiversity). It might also indi-
cate differences in tolerance towards benzene. The DGGE band rep-
resenting *Thioalkalivibrio* sp. K90 mix (d11, d13 and d14) becomes, for instance, dominant between day 33 and 47. During this time the effluent benzene concentrations were on average 2 times higher (26 ± 3 μM) compared to the rest of the experiment (data not shown).

Table 4 showed that different phylotypes of *Thioalkalivibrio* were present at different times during the reactor run (Fig. 3). A shift in dominance between different phyl-
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### 3.4. DGGE and clone library analysis

To investigate microbial population dynamics and overall diver-
sity in time, DGGE analysis was used of which 19 bands were
excised and sequenced (Fig. 3). Clone libraries of reactor samples

taken at day 1, 39 and 75 of Run 1 provided a more detailed view of
the microbial community (Figs. 4 and 5).

The DGGE gel showed different profiles over time with a chang-
ing degree of diversity in the reactor samples with the presence of
up to 4 dominant bands (Fig. 3). In general, the DGGE profile stayed
similar after day 20 indicating a stable community.

From the DGGE gel and the clone libraries it appeared that the bands and clones clustered within the α- and γ-Proteobacteria, Bacteriodetes, Clostridia and Bacillales (Figs. 3–5).

### 3.5. Benzene removal experiments

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The control experiment shows that in the absence of microor-
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Idiomarina have also been related to degradation of crude oil and diesel fuel at halophilic conditions (Kleinsteuber et al., 2006; Mnif et al., 2009). One of the few studies concerning mono-aromatic degradation under halo-alkaline conditions showed the biodegradation of catechol, which is a common intermediate in benzene biodegradation, by *Halomonas campisalis* (Alva and Peyton, 2003).

A distinct cluster within the *α*-Proteobacteria comprised of members related to the genera *Rhodobacter* and *Roseinatronobacter* (Fig. 4). Within these genera several bands (d6–8, d15, d17 and d18) in the DGGE and several clones from day 39 and 75 were closely related to *Rhodobaca barguzinensis* (Boldareva et al., 2008), *Roseinatronobacter thiooxidans* (Sorokin et al., 2008) and *Roseinatronobacter monicus* (Boldareva et al., 2007). Overall these bacteriochlorophyll *α* containing bacteria thrive in alkaline and/or (hyper) saline environments.

*R. Thiooxidans* and *R. monicus* are known to oxidize sulfur compounds such as sulfide, thiosulfate and elemental sulfur into sulfate using it as an additional energy source. In addition, several members of the *Halomonas* group are capable to partially oxidize sulfur compounds such as thiosulfate and sulfide into tetrathionate under halo-alkaline conditions. The produced tetrathionate can react with sulfide with the formation of sulfur and thiosulfate (Sorokin et al., 2008). Therefore, species related to the genus *Rhodobacter* and *Halomonas* present during the reactor experiments may have contributed directly or indirectly (through tetrathionate catalysis) to the complete sulfide conversion into sulfate as observed in the reactor runs.

Sequences closely related to the halophilic bacteria *Pseudomonas halophila* = *Halomonas variabilis* (Sorokin and Tindall, 2006) were only found in the clone library derived from day 1 (Fig. 4).
Within the *Bacteroidetes* band d12 and 19 clones from day 39 were found to be closely related to an uncultured *Lewinella*-like organism (CFB group bacterium; AF45299) from halo-alkaline Mono Lake in the USA (Figs. 3 and 5).

Some sequences from clone libraries day 1, 39 and 75 were found to be closely related to an alkaliphilic dissimilatory iron-reducing bacterium from the halo-alkaline Soap Lake (US) within the *Clostridia* (unpublished, Fig. 5).

Within the *Bacillales* (Fig. 5), several clones from day 39 and 75 could be divided in three distinct groups: (1) associated with *Alkalibacterium olivapovliticus* (Ntougias and Russell, 2001), (2) related to *Bacillus selenitireducens* (Switzer Blum et al., 1998) and (3) related to uncultured bacteria isolated from Mono Lake (AF454301) and from a hypersaline lake in Chili (Demergasso et al., 2008).

All heterotrophic microorganisms detected in the reactor biomass by molecular analysis were related to alkaliphilic and/or halophilic bacteria. However, the presence of heterotrophic organisms belonging to the *Clostridiales* and anaerobic members of *Bacillales* indicates that facultative anaerobic microorganisms were present in the bioreactor. Apparently small anaerobic niches are formed within the bioreactor possibly related to small areas (<0.1% of total volume) where solids were able to settle.

### 4. Conclusion

This study shows that it is possible to biologically treat sulfidic spent caustic solutions, originating from a refinery, at halo-alkaline conditions. Sulfide removal was complete up to 27 mmol L$^{-1}$ day$^{-1}$ by its conversion into sulfate. The sulfide conversion was accomplished by soda lake bacteria belonging to the genus *Thioalkalivibrio*. Benzene was removed by 93% in the biological reactor experiments. Calculations indicate that besides a high stripping efficiency also biodegradation of benzene took place. Heterotrophic organisms related to the genera *Marinobacter*, *Halomonas* and *Idiomarina* were shown to be present in the reactor and might have been involved in benzene biodegradation.

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### References


bacteriochlorophyll a-containing bacterium Roseinatronobacter monicus sp. nov. from the hypersaline Soda Mono Lake (California, United States). Microbiology 76, 82–92.


